CHAPTER I
GENERAL INTRODUCTION

1.1 Background

1.1.1 Aquaculture

Aquaculture has experienced significant growth in recent years due to the rising demand for fishery products and the depletion of wild fish stocks. With the global population increasing rapidly, the need for affordable protein sources has become crucial, making aquaculture a vital solution (El-Saadony et al., 2021). Aquaculture plays a critical role in food and nutrition security, provides livelihoods for millions of people, and it is one of the fastest-growing sectors in the world. In addition to increasing aquaculture production, there is a high demand for feed in the aquaculture sector. Compared to other animal food-production sectors, aquaculture growth is worth looking (Sidiq et al., 2024). Aquaculture allows the supply of fish having high protein levels, which has an important place in human nutrition, to the market as well as its contribution to the economy of a country. Societies, aware of the importance of balanced and healthy diets, frequently consume aquaculture products to meet their animal protein needs; therefore, healthy and high-quality aquaculture has gained great importance (Arshad et al., 2022).

Governments and development agencies are increasingly focusing on aquaculture as a promising activity to meet global food demands. It outpaces other food production methods in growth rate, making it essential for addressing food shortages and malnutrition (FAO, 2020). Successful aquaculture relies on maintaining water quality, providing proper nutrition, encouraging breeding, and preventing diseases and predators (Rastegari et al., 2023). However, challenges such as the high cost of fish feed ingredients and frequent disease outbreaks hinder the full potential of aquaculture's intensification and commercialization (Tabassum et al., 2021).

1.1.1.1 World aquaculture production

Aquaculture has become a crucial component of global food demands, playing a pivotal role in enhancing food security and nutrition. By 2018, it accounted over 82.1 million metric tons of fish, contributing to 46% of total fish production and fulfilling 52% of fish consumed by people worldwide. Projections indicate aquaculture production will surge to 109 million metric tons by 2030, surpassing capture fisheries in total output (Mair et al., 2023). Over the past three decades, aquaculture has maintained an impressive average growth rate of 6.7%, though this pace has moderated to 3.5% annually between 2016 and 2021. By 2021, total aquaculture output reached a record-breaking 126 million tonnes, with finfish as the top contributor at 47.1% (FAO, 2022).

Aquaculture production is rising internationally, and its contribution to overall fish supply (excluding aquatic plants) is bigger than catch fisheries in Asia-Pacific, where it accounts for around 60% of regional fish production. At the country level, except for Oceania, 39 countries today produce more aquatic animals through farming than from fishing (Bjørndal et al., 2024). The growth of aquaculture has been fueled by various factors, including the expansion of global trade, declining availability of wild fish, competitive product pricing, rising incomes, and urbanization. Aquaculture in Asia provided by far the most to global production of aquatic animals, whereas aquaculture in North America and Sub-Saharan Africa contributed 10.1 percent and 7%, respectively (Naylor et al., 2021).

1.1.1.2 Challenges facing aquaculture

The sustainability of aquaculture depends on many factors, including site selection, seed quality, disease outbreak, water quality, and cost-effective feed production. Recent studies showed that the majority of farm production costs mainly come from feed production, accounting for about 66% to 84% of total production costs, making it essential to reduce feed expenses to ensure profitability (Jahangiri & Esteban, 2018). To address this concern, researchers have increasingly focused on developing feed additives, particularly prebiotics, probiotics, and their combination (synbiotics) to enhance feed efficiency in aquaculture. Incorporating these additives into aquafeed has shown promising results in improving food value, digestive enzymes, growth, and immune responses in fish (Huynh et al., 2017). Probiotics, prebiotics, and synbiotics have emerged as practical, safe, and ecologically acceptable solutions for improving tilapia production by enhancing growth performance, feed utilization, immunity, disease resistance, and fish survival against infections and environmental stress. Overall, it has been discovered that using them in fish diets or raising water improves the well-being of fish in aquaculture (Mugwanya et al., 2022). Among the species benefiting from these strategies, Nile tilapia (*Oreochromis niloticus*) stands out as a prominent choice due to its numerous attributes that make it an ideal candidate for aquaculture practices.

1.1.2 Nile tilapia

The Nile tilapia (*Oreochromis niloticus*) (Figure 1.1) is one of the most important freshwater aquaculture species (Li et al., 2022). They have many attributes that make them a great choice for aquaculture, including their fast growth, tolerance to a wide range of environmental conditions, resistance to stress and disease, ability to reproduce in captivity, feeding on low trophic levels, and acceptance of artificial feeds immediately after yolk-sac absorption (El-Sayed, 2020).

Nile tilapia stands out as one of the most extensively cultivated fish species, playing a crucial role in ensuring food security by providing an affordable source of fish for those with limited resources (Eissa et al., 2022). Within intensive rearing systems, enhancing profits necessitates access to cost-effective and sustainable feed ingredients that can effectively support the growth and welfare of the fish (Allam et al., 2020; El-Ouny et al., 2023). Recognized as the most widely cultured economic fish species globally (Hai, 2015), tilapia production has witnessed a fourfold increase over the past decade (Amin et al., 2019). Consequently, a series of studies have focused on how to increase yields (Amin et al., 2019), enhance product quality (Feitosa et al., 2017), and mitigate significant economic losses attributed to disease infections (Dong et al., 2017).

Figure 1. 1 Nile tilapia (*Oreochromis niloticus)*

The global production of farmed Nile tilapia has seen significant growth from 1980 to 2022 (Fig 1. 2), establishing it as the third most abundantly farmed finfish species worldwide. This increase is attributed to its adaptability, disease resistance, and the development of improved aquaculture techniques. Nile tilapia accounts for approximately 75% of total tilapia production, contributing about 8.3% to global aquaculture (Samaddar et al., 2024). In 2022, global production reached over 5 million tonnes (Debnath et al., 2023).

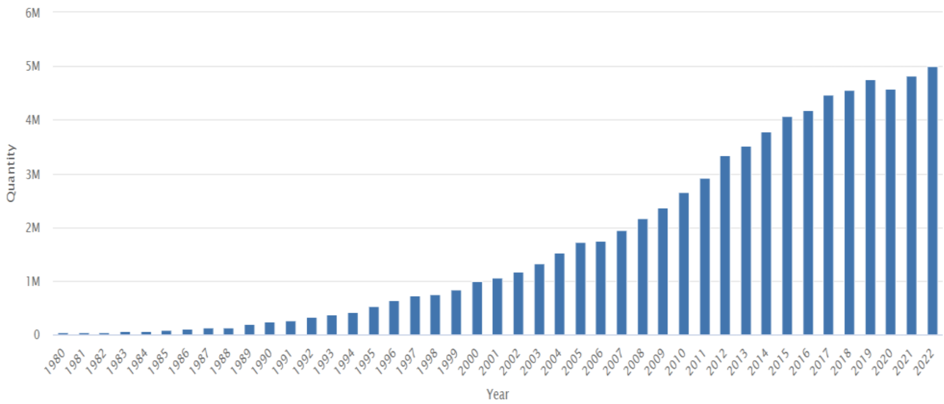


Figure 1. 2 Global production of farmed tilapia 1980-2022
Source: FAO FishStatJ database (https://www.fao.org/fishery/en/aqspecies/3217/en).

1.1.2.1 Natural distribution and adaptability

Nile tilapia are naturally distributed in the Nilo-Sudanian region, Ethiopian Rift Valley, the western Rift Lakes (Lake Albert, Lake Edward, Lake George, Lake Kivu and Lake Tanganyika) and Lake Turkana in the eastern Rift Valley. Nile tilapia is also naturally established in Central and Western Africa (Senegal, Gambia, Volta, Niger, Benue and Chad river basins) (Figure 1.3 a) (El-Sayed & Fitzsimmons, 2023). Nile tilapia are highly adapted to tropical, subtropical and temperate environments. They are characterized by their fast growth rates, tolerance to extreme environmental conditions (such as temperature, salinity, pH, and low dissolved oxygen), high resistance to stress and diseases, trophic plasticity and feeding on low trophic levels, and their ability to reproduce in captivity (Assefa & Getahun, 2015). These attributes made them an ideal candidate for aquaculture all over the globe. It is no surprise, therefore, that 114 Nile tilapia introductions have been recorded worldwide (Figure 1.3 b), mainly for aquaculture and fisheries enhancement (Deines et al., 2016).

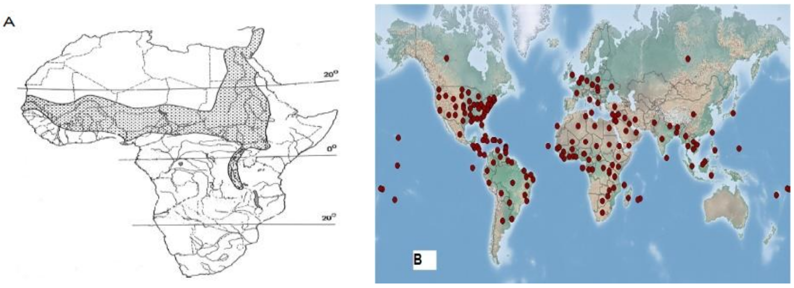


Figure 1. 3: a: Natural distribution of Nile tilapia in Africa. b: Global Nile tilapia introductions
Source: (El-Sayed & Fitzsimmons, 2023)

1.1.2.2 Environmental tolerance ranges

Nile tilapia (*Oreochromis niloticus*) is recognized for its remarkable tolerance and adaptability to varying environmental conditions, making it a preferred species in aquaculture. This species can survive in temperatures ranging from 11 to 42°C, with optimal growth occurring above 16°C (Palmer et al., 2024). Additionally, Nile tilapia can endure low dissolved oxygen levels, tolerating concentrations as low as 3 to 4 mg/l (Samaddar et al., 2024).

The water quality characteristics of pH and ammonia are critical for optimal tilapia culture. While tilapia can tolerate a wide pH range, the ideal growth occurs between pH 7 to 9, with studies indicating that even slight acidification does not adversely affect growth rates (Rebouças et al., 2022). Ammonia levels are particularly significant, as concentrations above 0.1 mg/L can depress feed intake and growth, with toxicity thresholds for blue and Nile tilapia at 2.5 and 7.1 mg/L, respectively (Abd El-Hack et al., 2022). Limits and optimum of water quality parameters for tilapia are presented in (Table 1.1).

Table 1. 1 Limits and optimum water quality parameters for tilapia

Parameters	Range	Optimum for growth	Reference
Dissolved oxygen, mg/L	Down to 0.1	> 3	(Samaddar et al., 2024)
Temperature °C	8 to 42	22 to 29	(Palmer et al., 2024)
pH	3.7 to 11	7 to 9	(Rebouças et al., 2022)
Ammonia, mg/L	Up to 7.1	< 0.05	(Abd El-Hack et al., 2022)

1.1.2.3 Nutritional requirements of Nile tilapia

Nile tilapia (*Oreochromis niloticus*) exhibit varying nutritional requirements across different life stages, influenced by size, age, and dietary composition. Early juveniles (0.02-10.0 g) require diets with

25-30% protein, essential amino acids, and low carbohydrates to optimize growth (Tippayadara et al., 2022; Meurer et al., 2024), while sub-adults (10-25 g) shift towards diets with increased energy from lipids and carbohydrates, reducing protein levels to around 25% (Huang et al., 2022). In contrast, adults (>25.0 g) can thrive on diets containing as low as 26.45% protein, utilizing higher carbohydrate levels for energy (El-Sayed, 2020). This transition in dietary needs is crucial for maximizing growth and metabolic efficiency. The balance of proteins, lipids, and carbohydrates is essential for health and performance; excessive protein can lead to inefficiencies and increased aquaculture costs. Lipid requirements are also significant, with a minimum of 5% lipids recommended, while diets containing 10-15% lipids enhance growth and protein utilization efficiency. Both n-3 and n-6 polyunsaturated fatty acids are vital for optimal growth in hybrid tilapia. Carbohydrates serve as a cost-effective energy source, with tilapia efficiently utilizing 35-40% digestible carbohydrates. Additionally, vitamin requirements, especially vitamin E, are influenced by dietary lipid levels, necessitating careful feed formulation to ensure adequate nutrient intake for optimal growth and health (Furuya et al., 2023). The major nutrient requirements of cultured tilapia are presented in Table 1.2.

Table 1. 2 Nile tilapia dietary requirements according to size

Fish size	Fry (<10g)	Fingerling (10–30g)	Grow out (>30g)	>300g	Broodstock
Crude protein	40-45	28-35	25-30	20-25	40-45
Crude lipid	6-13	6-13	4-12	4-12	6-12
Crude fiber	<4	<8	<8	<8	<10
Ash	<16	<16	<16	<16	<16
Carbohydrate	25-30	25-30	25-30	25-30	25-30
Moisture	<10	<10	<10	<10	<10

Source: (FAO, 2015)

1.1.2.4 Feeding rates

Feeding rate and frequency are generally influenced by various factors, including feeding behavior, physiology, feed quality, and environmental conditions. provides Recommended feeding rates and frequencies for complete feed tilapia at their optimal growth temperature presented in (Table 1.3).

Table 1. 3 Feed rates based on tilapia body size

Average fish size (g)	Jauncey (1998)		NRC (2011)	
	Feed rate (%)	Frequency (day^{-1})	Feed rate (%)	Frequency (day^{-1})
<50	7 to 6.5	3 – 6	4.5	3
50	3	3	3.7	3
Up to 100	2	2	3.2	3
Up to 200	2	2	2.8	2
Up to 300	1.8 to 1.5	2	2.3	2
>300	-	-	2.0 to 1.4	2

Feeding rates are given as % of total tilapia biomass per day

Source: (Jauncey, 1998)

1.1.3 Prebiotics

A prebiotic refers to a substance, such as a substrate, long chain sugar, nutrient, or fiber, that functions as nourishment for the advantageous bacteria residing within the digestive tract of a host (Mountzouris, 2022). Moreover, a prebiotic can be characterized as a substance that exhibits resistance to the acidic conditions prevailing in the stomach, possesses the ability to undergo fermentation by the gut microbiota, and facilitates the proliferation of gut microbiota, hence enhancing the overall well-being of the host (Davani-Davari et al., 2019). Tran et al. (2022), have indicated that prebiotics serve as energy sources for gut microbes, hence enhancing health outcomes in the host. In general, prebiotics can be described as complex carbohydrates with long chains that serve as a source of energy for beneficial microorganisms with the aim of improving the overall health of an organism. The primary origins of prebiotics are predominantly sourced from botanical sources (Mohammadi et al., 2020). Additionally, edible mushrooms have been identified as a source of prebiotics (Singarayar et al., 2024), whereas animal dairy products contribute to a smaller proportion of prebiotic sources. Prebiotics, essential for maintaining a healthy gut microbiome, are abundantly found in a diverse range of natural food sources. These compounds, which include vegetables, fruits, beans, seaweed, microalgae, and animal milk, provide a rich and accessible source of prebiotics that can be easily incorporated into a balanced diet (Ahmadifar et al., 2019; Elumalai et al., 2020).

Prebiotics in Nile tilapia farming have been found to have several beneficial effects. They enhance growth by fostering the proliferation of beneficial gut bacteria, which improves nutrient absorption and utilization, ultimately resulting in better growth performance (Ramos et al., 2017). Prebiotics have been shown to boost the immune system of Nile tilapia, thereby increasing their resistance to diseases and infections (Selim and Reda, 2015; Li et al., 2019). The incorporation of prebiotics in fish farming can minimize the need for antibiotics by promoting fish health and reducing the likelihood of disease outbreaks, thereby contributing to a more sustainable and responsible aquaculture practice (Abdul-Kari et al., 2021). Prebiotics play a crucial role in enhancing the health of Nile tilapia by fostering the growth of beneficial gut bacteria. This leads to a balanced gut microbiome, which in turn contributes to improved overall health and productivity in fish farming. Additionally, incorporating prebiotics into Nile tilapia diets can be economically beneficial due to enhanced growth performance, reduced feed consumption, and increased resistance to pathogens (Dias et al., 2019) as shown in (Figure 1.4).

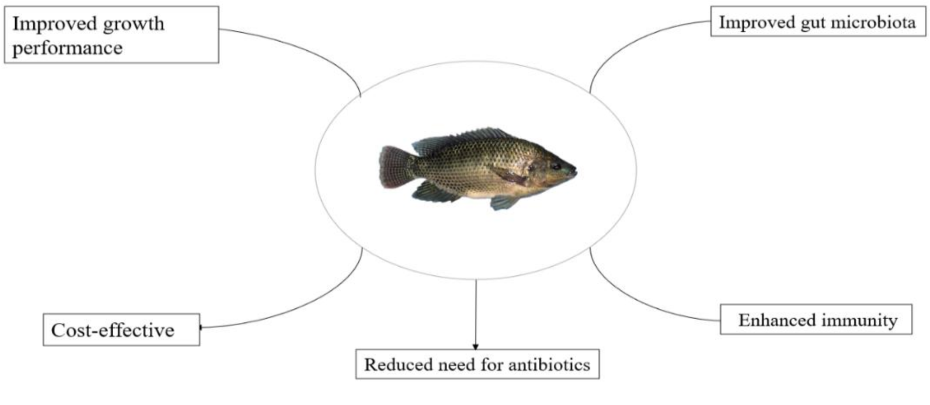


Figure 1. 4 Positive effects of prebiotic on Nile tilapia Farming

1.1.3.1 Prebiotics modes of action in tilapia

1.1.3.1.1 Nurturing beneficial gut microbes through selective fermentation

Prebiotics are essential in tilapia farming, as they provide a nutrient-rich environment for beneficial gut bacteria, which in turn, significantly impact the health and growth of the aquatic organisms (Wee et al., 2022). One of the key mechanisms underlying their effectiveness is their role as a nutrient source for specific bacteria in the gastrointestinal tract (GIT). Notably, prebiotics like inulin undergo selective fermentation by microbes such as Bifidobacterium, leading to the secretion of enzymes like beta-fructosidases that degrade inulin's glycosidic bond. This fermentation process alters the gut microbial composition, favoring the proliferation of beneficial microbes over pathogens (Merrifeld et al., 2010). Studies on tilapia have shown that supplementing diets with specific prebiotics, such as glucan, leads to increased populations of beneficial firmicutes and proteobacteria in the gut, which subsequently contribute to improved growth and health (Souza et al., 2020).

1.1.3.1.2 Stimulation of digestive enzyme production via microbial proliferation

Prebiotics also exert their impact on tilapia farming by indirectly stimulating the production of essential digestive enzymes. The proliferation of beneficial gut microbes, promoted by prebiotics, results in increased secretion of these enzymes, thereby enhancing the fish's ability to digest and absorb nutrients. Recent research by Aryati et al. (2021), demonstrated that incorporating honey, a prebiotic, into *O. niloticus* diets led to a higher population of gut microbes that externally produce crucial digestive enzymes like amylase, protease, and lipase. This positive correlation between prebiotic supplementation and enzyme activity translates into improved fish growth performance. This phenomenon is consistent with findings by Abd El-latif et al. (2015), who highlighted the role of prebiotics in enhancing digestive enzyme activity and production in tilapia. Enhanced enzyme activity not only supports nutrient utilization but also positively correlates with overall body weight and feed efficiency (Hoseinifar et al., 2015).

1.1.3.1.3 Increasing disease resistance and the innate immune system

Prebiotics have a crucial impact on the innate immune system of tilapia in aquaculture, significantly boosting its resilience and overall health, while also promoting the maintenance of gut microbial equilibrium. The immune system activation and protection against possible infections have been associated with the presence of beneficial gut bacteria, which is regulated by prebiotics. The research conducted by Akhter et al. (2015) revealed that prebiotics can enhance the innate immune response in fish and shellfish, hence providing them with increased resistance against infections. Moreover, the diverse range of activities exhibited by prebiotics encompasses the secretion of extracellular enzymes produced by bacteria and the production of bioactive metabolites. These compounds, as shown by Huynh et al. (2017), can indirectly influence tilapia growth and health, contributing to a comprehensive strategy for optimizing growth, reproduction, immunity, and disease resistance efficiency (Hoseinifar et al., 2015).

1.1.3.2 Application of prebiotics in Nile tilapia farming

The utilization of prebiotics in tilapia aquaculture holds significant promise, as it offers a multitude of potential advantages such as enhanced growth performance, enhanced immune response, diminished reliance on antibiotics, improved gut health, and heightened resistance against diseases. Prebiotics commonly utilized in aquaculture encompass a range of substances such as β-glucan, inulin, arabinoxylan oligosaccharide (AXOS), mannan oligosaccharide (MOS), galacto-oligosaccharide (GOS), fructo-oligosaccharides (FOS), and oligosaccharides. Prebiotics have been extensively studied and are known to offer numerous advantages in aquaculture. Research indicates that they can significantly boost growth performance (Li et al., 2019), enhance feed efficiency (Shoaei et al., 2015), strengthen the immune system (Li et al., 2021), and improve disease resistance (Abdel-Latif et al., 2022; Li et al., 2018; Yilmaz et al., 2022). As a result, the incorporation of prebiotics into aquaculture methodologies is expected to augment the output of species. Nevertheless, it is crucial to acknowledge that the reaction of the host gut microbiota to prebiotics exhibits selectivity (Gibson et al. 2017). Prebiotics like fructooligosaccharides (FOS) and galactooligosaccharides (GOS) have been observed to selectively promote the growth of specific gut microbiota, such as Lactobacillus and Bifidobacterium species, due to their ability to be fermented by these bacteria, enhancing their enzymatic activity (Gibson et al., 2017). These prebiotics play a crucial role in modulating the gut microbiota by stimulating the proliferation of beneficial bacteria while inhibiting the growth of pathogenic strains (Kaewarsar et al., 2023). The fermentation of FOS and GOS by probiotic bacteria leads to the production of short-chain fatty acids (SCFA), which have various health benefits and contribute to the overall metabolic activity of the gut (Hadinia et al., 2022). Therefore, the preference of certain gut microbiota for FOS and GOS may be attributed to the specific enzymatic processes involved in fermenting these prebiotics, highlighting the importance of understanding the interactions between prebiotics and gut bacteria for promoting gut health and overall well-being. However, the incorporation of supplementary prebiotics into aquaculture diet has the potential to broaden the range of gut microbiota in aquatic organisms, hence promoting improvements in their overall health.

The application of prebiotics in Nile tilapia has been ongoing since 2010 and has been increasing every year. his is achieved by fostering the proliferation of beneficial gut bacteria, thereby strengthening the fish's immune system and increasing their resistance to pathogens (Figure 1.5).

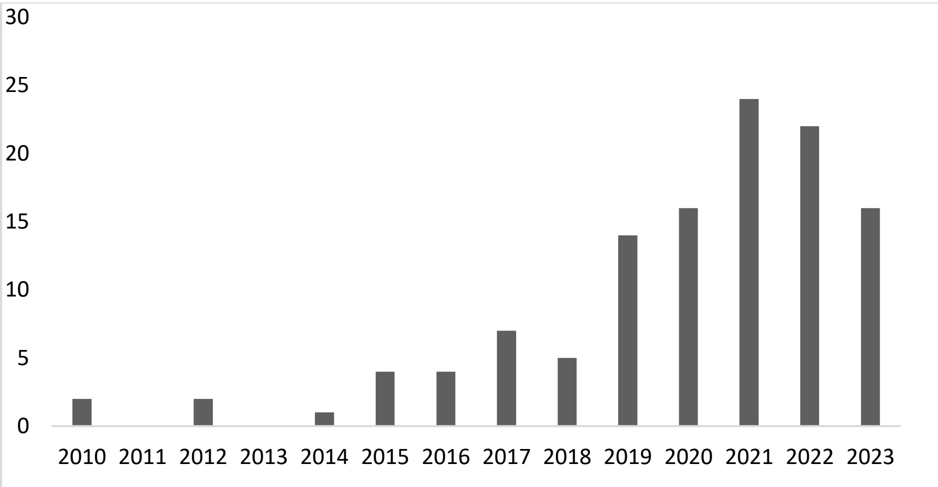


Figure 1. 5 Application of prebiotics in Nile tilapia farming 2010 – 2023

Table 1.4 provides a summary of prebiotic applications in Nile tilapia farming, their dosages, durations, and the responses observed in Nile tilapia. These findings suggest that prebiotics can have significant positive effects on the health and performance of Nile tilapia, including improved growth, immune function, and resistance to pathogens. However, the optimal dosage and duration of prebiotic use may vary depending on the specific prebiotic and the fish species.

Table 1. 4 Summary of prebiotic applications in Nile tilapia farming

Prebiotics	Dosage	Duration	Response	Reference
Inulin	5g kg^{-1}	1 and 2 months	Hematocrit ↑, NBT (Superoxide activity) ↑, lysozyme activity ↑, Protection from A. hydrophila challenge ↑.	Ibrahem et al., 2010
Inulin	15–20 g.kg^{-1}	60 days	intestine and glycogen deposition in liver ↑, immune parameters ↑, Haematological parameters ↑, Biochemical parameters ↑	Ali et al., 2017
Inulin	5 g g.kg^{-1}	8 weeks	growth performance ↑, blood chemicals parameter↑, lysozyme activity ↑, ACH50 activity ↑	Tiengtam et al., 2015
Inulin	2.5 g.kg^{-1}	2 weeks	Growth performance ↑, Immuno- haematological indices ↑, liver and spleen structure ↑, parasitic infection ↓	Yones et al., 2020
β-glucans (BG01 & BG02)	0.1 g.kg^{-1}BG01 & 0.1 g kg^{-1} BG02	30 days	Growth performance ↑, survival rate after bacterial infection ↑	Pilarski et al., 2017
MOS	0.5 %	12 weeks	Weight gain ↑, FCR ↓, lysozyme activity ↑, Mortality rates against *A. hydrophila* ↓	Kishawy et al., 2020
MOS	4 g g.kg^{-1}	16 weeks	Growth performance ↑, serum albumin ↑, total protein ↑, RBCs ↑, and Hb levels in lead-contaminated diets ↑	Ayyat et al., 2020

FOS	2% & 3 %	8 weeks	Final weight ↑, weight gain ↑, SGR ↑, FCR ↓, intestinal enzymes activities ↑, histological feature of intestinal villi ↑	Abd El-latif et al., 2015
FOS	2 & 4 $g.kg^{-1}$	8 weeks	Weight gain ↑, FCR ↓, digestive enzyme activity ↑, protein and lipid retention ↑.	Poolsawat et al., 2020
β-glucan + Mos	1.5 & 3 $g.kg^{-1}$	60 days	Growth ↑, feed utilization ↑, FCR ↓, Villus height ↑, goblet cells ↑, intraepithelial lymphocytes ↑, body composition ↑, serum total protein ↑, albumin ↑, globulin ↑	Selim and Reda, 2015
β-glucan	0.1% of diet	30–45 days	Growth ↑, immune performance ↑.	Koch et al., 2021

1.1.4 Probiotics

Probiotics refer to live microorganisms that are introduced into the gastrointestinal tract (GIT) through food or water with the aim of improving the internal microbial balance and thus supporting overall well-being (Gatesoupe, 1999; Azad et al., 2019). The utilization of probiotics in aquaculture has been recognized as an ecologically sustainable method for prevention of disease outbreaks, enhanced growth, and improved digestion (Guo et al., 2016; Adel et al., 2017).

Probiotics have been demonstrated to offer several positive effects on Nile tilapia farming. These benefits encompass improved immunological responses, enhanced growth, increased resistance to ammonia, better digestive health, and reduced stress levels. Probiotics can enhance the immunological responses of Nile tilapia, leading to a stronger immune system that helps the fish better resist diseases and infections, resulting in improved survival rates and overall health (Shija et al., 2023). Furthermore, probiotics can improve the growth rate of Nile tilapia by promoting the growth of beneficial gut microbes, which, in turn, support the overall health and development of the fish (Zabidi et al., 2021). They also aid in increasing the fish's resistance to ammonia, making them more resilient to the stressors of aquaculture environments (Cavalcante et al., 2020). Additionally, probiotics can enhance the digestive health of Nile tilapia by boosting the activity of digestive enzymes, which is crucial for the efficient breakdown of feed and the overall health of the fish (Xia et al., 2019). The use of probiotics also can help reduce stress levels in Nile tilapia, which is particularly important in aquaculture settings where high stress levels can lead to decreased growth rates and increased susceptibility to diseases (Munni et al., 2023). The Positive effects of probiotic on Nile tilapia Farming presented in Figure 1.6.

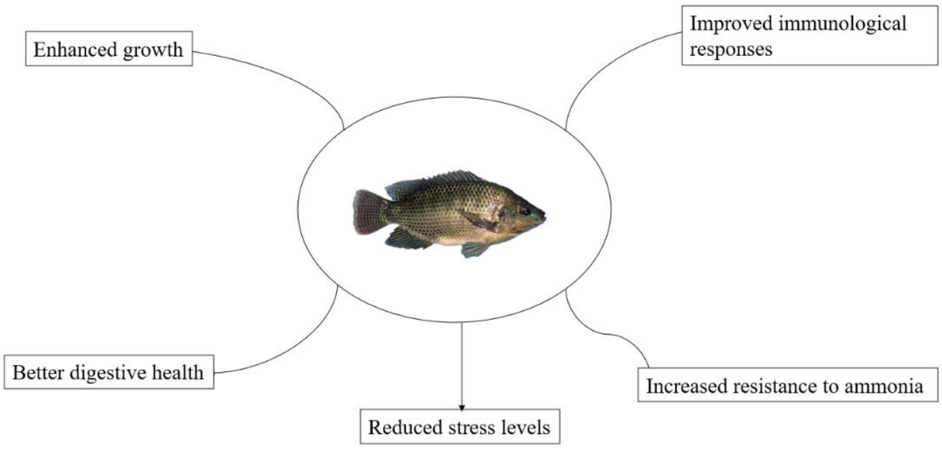


Figure 1. 6 Positive effects of probiotic on Nile tilapia Farming

1.1.4.1 Single and multi-spesies of probiotics in aquaculture

Probiotics can be added to fish feed singly or in combination between two or more in different forms such as multi-species probiotics (MSP), probiotics with plant extract, and probiotics with yeast extract (Subedi & Shrestha, 2020). The use of a single probiotic was the focus of the majority of studies on probiotics in aquaculture; however, a combination of probiotics is more beneficial. Multi-spesies probiotics provide more benefits for aquatic organisms because they provide a different synergistic effect than the use of single-strain probiotics (SSP). The effectiveness of MSP is due to a positive synergism relationship between species which leads to symbiosis (Puvanasundram et al., 2021). These probiotics can be used to increase the immune response and increase fish resistance to disease (Ringø, 2020). Probiotics can also increase beneficial intestinal microbial populations, intestinal morphology, and increase the activity of digestive enzymes that help increase nutrient absorption and feed utilization (Hai, 2015; El-Saadony et al., 2021).

The overarching concept behind the utilization of multi-species probiotics (MSP) is that although a specific single-strain probiotic (SSP) may have positive effects on a limited number of health domains, the incorporation of many species has the potential to expand the range of health benefits (Ouwehand et al., 2018). For example, the utilization of *Lactobacillus plantarum* has demonstrated the ability to mitigate disease symptoms, while *Lactococcus lactis* has been shown to enhance immunity against *Streptococcus iniae* infections in Olive flounder (*Paralichthys olivaceus*) (Beck et al., 2015). Therefore, the utilization of these two species of probiotic bacteria mutually enhances one another in order to expand the range of benefits experienced by the host organism. The understanding that a certain strain exhibits distinct and occasionally exclusive biological impacts provides valuable insights for the advancement of personalized medical treatment tailored to individual cases or pathologies (Lin et al., 2017).

The limitations of single-strain probiotics (SSPs) in adhering to intestinal mucosa, surviving the harsh gastrointestinal environment, and establishing a stable probiotic niche underscore the need for multi-species probiotics (MSPs). Unlike SSPs, MSPs capitalize on the synergistic interactions among diverse species, enhancing their ability to colonize, produce enzymes, and support host metabolic functions. This combination of species offers cumulative health benefits by leveraging their distinct characteristics, which SSPs often lack. Emerging evidence highlights the superior efficacy of MSPs in overcoming challenges such as low viability and limited mucosal adherence, leading to improved probiotic activity and host health outcomes (Liang et al., 2023; Han et al., 2024; Zhao et al., 2024). While the exact mechanisms behind these interactions are not fully understood, ongoing research continues to explore their potential (Fentie et al., 2024).

The utilization of a microorganism mix as a fermenter is highly advantageous due to its composition of natural ingredients, which contain superior microbes that can produce a variety of beneficial enzymes. Aslamyah et al. (2018) provide a description of microorganisms exhibiting mixed characteristics. The composition comprises a combination of bacteria, fungi, yeast, and molds that synthesize enzymes of significance in the process of fermenting raw materials, thus augmenting the nutritious composition of milkfish feed.

Bacillus sp are widely regarded as superior probiotics in aquaculture due to their unique characteristics, including the ability to produce antimicrobial substances, enhance immune responses, and their sporulation capability, which ensures heat tolerance and extended shelf life (Kavitha et al., 2018). These traits allow *Bacillus* sp to thrive in diverse environments, making them effective in improving fish health and performance. They boost digestive and antioxidant enzyme activities, upregulate immune-related and stress-related gene expression, and increase resistance to pathogens (Liu et al., 2017). These benefits result in improved feed utilization and growth rates in various fish species, such as Nile tilapia and grass carp, as well as crustaceans like freshwater prawn (Gupta et al., 2016). *Bacillus* probiotics enhance digestive enzyme activities, including protease, amylase, trypsin, and lipase, which are critical for nutrient metabolism and feed efficiency (Dawood & Koshio, 2016). They may either synthesize enzymes directly or stimulate endogenous enzyme production in fish (Dawood et al., 2016). For instance, dietary *Bacillus subtilis* has been shown to significantly increase digestive enzyme activity in Nile tilapia, yellow river carp, and grass carp, leading to improved feed digestibility and growth (Fan et al., 2018). These properties make *Bacillus* species a valuable tool in sustainable aquaculture, reducing production costs and promoting fish health and welfare (Kuebutornye et al., 2019).

Saccharomyces sp., a commonly used probiotic in aquafeeds, is valued for its functional components such as β-glucans, nucleic acids, mannan oligosaccharides, and chitin (Dawood & Koshio, 2019). It produces a variety of metabolites, including enzymes, oligosaccharides, amino acids, peptides, organic acids, and vitamins, which play essential roles in enhancing fish health and performance (Hossain et al., 2020). These nutrients and immunostimulant constituents have been shown to improve growth performance, immune response, and disease resistance in fish and crustaceans (Zhang et al., 2018). Additionally, *Saccharomyces* sp. contributes to cytokine modulation and improves gastrointestinal health in aquatic animals (Huyben et al., 2017). The supplementation of *Saccharomyces* sp. in aquafeeds has been proven to enhance growth, immunity, and disease resistance across various fish species. *S. cerevisiae*-fermented palm seed meal positively impacted digestive enzyme activity, intestinal health, immune functions, and growth performance in Nile tilapia (Dawood et al., 2020).

Rhizopus sp., a protein-rich filamentous fungus with an amino acid profile comparable to fish meal, has shown potential as a protein source in aquafeeds. Studies have indicated that fish fed with *Rhizopus oryzae* exhibited enhanced gut integrity, with higher trans-epithelial resistance (TER) in the distal intestine compared to those fed fish meal, suggesting a positive effect of *Rhizopus* metabolites on intestinal health (Karimi et al., 2021). Additionally, *R. oryzae* produces various enzymes, including cellulase, xylanase, pectinase, amylase, protease, and lipase, which enhance the breakdown of cellulose, hemicellulose, pectin, proteins, and fats, contributing to improved nutrient bioavailability (Oktapiani et al., 2021). *Rhizopus sp.* also demonstrates antimicrobial and antioxidant properties, making it effective against pathogenic bacteria and beneficial for overall fish health. Moreover, it can convert inorganic minerals into organic forms, enhancing mineral absorption and utilization (Endrawati & Kusumaningtyas, 2018). Fermented feeds using *Rhizopus sp.* have been reported to yield better results than non-fermented feeds, showcasing its potential as a feed additive for improving the growth and health of aquatic species.

The combination of *Bacillus* spp., *Saccharomyces* sp., and *Rhizopus* sp. as a multi-species probiotic creates a powerful synergy that significantly enhances fish health and performance compared to using single-species probiotics. Each species brings unique strengths: *Bacillus* sp. boosts digestive enzyme activity, strengthens immunity, and provides long-lasting stability; *Saccharomyces* sp. contributes bioactive compounds that enhance growth, immune response, and gut health; and *Rhizopus* sp. produces enzymes and bioactive substances that improve nutrient absorption and gut integrity.

1.1.4.2 Probiotics mode of action in tilapia

The use of probiotics in tilapia aquaculture is employed with the intention of stimulating growth and bolstering the tilapia's ability to withstand diseases (Shija et al., 2023). While the specific mechanism through which probiotics affect fish is still not fully understood, studies have demonstrated that probiotics can produce varying effects that are specific to the host and the type of probiotic used (Hai, 2015). Several potential mechanisms of action of probiotics in tilapia aquaculture can be identified, including:

1.1.4.2.1 Competition for nutrients

In general, bacteria have a requirement for iron, which is recognized as one of the vital minerals necessary for their growth. However, the growth of bacteria is hindered due to the restricted availability of iron in animal tissues and fluids. Siderophores that bind to iron make it easier for bacteria to get the iron they need to grow (Mugwanya et al., 2022). Gram et al. (1999) earlier posited a correlation between the synthesis of siderophores and the pathogenicity of specific microorganisms. Bacteria that possess the ability to produce siderophores exhibit the capacity to sequester iron in an environment characterized by low iron levels. This sequestration process is a challenge for pathogenic bacteria, as it hinders their ability to absorb iron and thus inhibits their growth. Furthermore, it has been observed that some probiotic microorganisms have the ability to produce digestive enzymes, which can effectively diminish the presence of anti-nutrient compounds in feed and enhance the absorption of nutrients. Consequently, this leads to an improvement in feed utilization and the overall performance of aquatic organisms (Steinfeld et al., 2015). Carbohydrase's, lipases, and proteases are enzyme types that have been identified in previous research (Eshaghzadeh et al., 2015). Taoka et al. (2007), reported that the administration of probiotics (*Bacillus subtilis*, *Lactobacillus acidophilus*, *Clostridium butyricum*, and *S. cerevisiae*) directly or by dietary inclusion resulted in enhanced activity of digestive enzymes in tilapia. In their study, Tan et al. (2019) reported an observed augmentation in digestive enzyme activity in *O.*

niloticus when subjected to nutritional supplementation of 10^6 and 10^7 CFU g^{-1} of *Rumeliibacillus stabekisii*.

1.1.4.2.2 Effect of probiotics on intestinal morphology

According to Nayak (2010), it is believed that probiotics have the ability to establish themselves and exert their advantageous effects within the intestinal tract of animals, which is recognized as a multifaceted ecosystem comprising non-pathogenic, pathogenic, and commensal bacteria. The growth of the intestines is facilitated by the presence of beneficial endogenous intestinal microbiota, which serve as crucial elements in the regulation of mucosal development and tolerance (Akhter et al., 2015). Probiotics are capable of retaining the healthy intestinal condition by reducing the amount of harmful pathogenic microorganisms that persist within the GI tract of fish and which can develop the structure of the intestinal epithelial layer by lowering the mucosal damage and increasing of absorption of nutrients (Merrifield et al., 2010).

1.1.4.2.3 Production of inhibitory substances

Probiotics are known to produce a variety of inhibitory compounds that exhibit antimicrobial and antiviral properties. The compounds produced by beneficial gut bacteria in Nile tilapia include a diverse array of substances that collectively contribute to the fish's overall health. These substances include siderophores, bacteriocins, hydrogen peroxide, lysozymes, proteases, volatile fatty acids (such as lactic, propionic, acetic, and butyric acid), organic acids, and extracellular enzymes. The production of these compounds is significant, particularly in the context of aquatic animals, where they are thought to play a crucial role in suppressing viral replication (Chauhan & Singh, 2019). Furthermore, inhibitory compounds lower the pH of the GIT, inhibiting the growth of opportunistic infections (Tinh et al., 2008). Extracellular compounds produced by *L. acidophilus* were found to have excellent antibacterial activity against *A. hydrophila* and *Streptococcus agalactiae* in vitro, according to Villamil et al. (2014). This could explain why *O. niloticus* developed better resistance to fish infections after consuming probiotic *L. acidophilus.*

1.1.4.3 Application of probiotics in Nile tilapia farming

Probiotics have garnered substantial interest in the aquaculture industry due to their multifaceted advantages. As dietary additives, they offer a high level of security and contribute to improved growth rates in fish. Additionally, probiotics enhance nutrient digestion, boost the host's immunity, and modulate the balance of beneficial gut bacteria. This balanced gut microbiota, in turn, enhances the overall health of the fish. Furthermore, the application of probiotics in aquaculture has been demonstrated to have a profound impact on the environment by significantly reducing the reliance on antibiotics and fostering a healthier aquatic ecosystem (El-Saadony et al., 2021). Probiotics emerge as a highly promising and environmentally friendly alternative to antibiotics, offering a safe and sustainable solution for aquaculture (Assefa & Abunna, 2018). The primary probiotics used in this context include *Bacillus*, *Lactobacillus*, *Enterococcus*, and *Saccharomyces*, which have been extensively studied and classified as effective and non-polluting agents (Anokyewaa et al., 2021). Probiotics function in the host's intestine to enhance growth and digestion by either secreting digestive enzymes themselves or stimulating the production of such enzymes (Nunes, 2018); Additionally, they inhibit the adhesion and growth of pathogens, maintain the balance of beneficial gut bacteria, and strengthen the mucosal barrier. These actions also modulate the immune response and antioxidant levels, ultimately enhancing the host's resistance to disease (Amoah et al., 2019).

The application of probiotics in Nile tilapia has been ongoing since 2008, and the use of probiotics has been increasing every year (Figure 1.7). There are numerous publications related to the use of probiotics in Nile tilapia, which optimize reproductive performance and result in economic advantages. Bacillus subtilis is one of the probiotic species that has been used in Nile tilapia, and it has been shown to improve growth performance and be profitable when applied by fish farmers. Studies have delved into the effects of probiotic supplementation on the growth, gut health, and disease resistance of juvenile Nile tilapia, revealing significant and promising results. Specifically, studies have shown improvements in weight gain, feed conversion ratio, and resistance to pathogens such as Streptococcus agalactiae, indicating the potential benefits of probiotics in enhancing the health and well-being of Nile tilapia. The use of probiotics in Nile tilapia is expected to continue to increase, as they provide benefits in terms of growth, health, and disease resistance, leading to improved survival and overall performance in aquaculture.

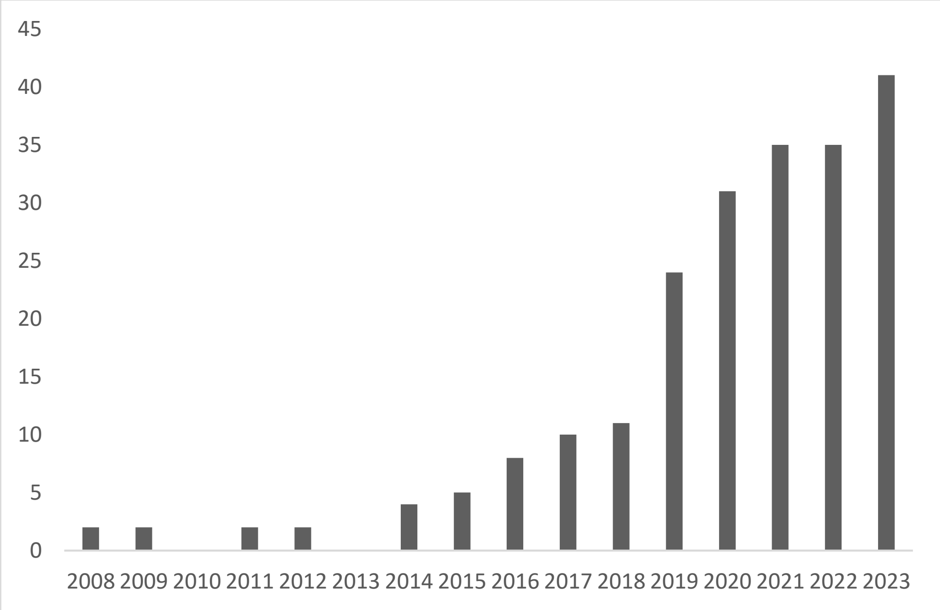


Figure 1. 7 Application of Probiotics in Nile tilapia Farming 2008 – 2023

Table 1.5 provides a summary about the application of probiotics in Nile tilapia farming, their dosage, duration, and response of various probiotics used in tilapia farming. These findings suggest that probiotics can have a beneficial impact on the growth and health of Nile tilapia.

Table 1. 5 Summary of probiotic applications in Nile tilapia farming

Probiotics	**Dosage**	**Duration**	**Response**	**Reference**
Bacillus pumilus	1x10^8 and 10^9 CFU/kg	4 weeks	Weight gain ↑, SR ↑, FCR ↓, Disease resistance against *S. agalactiae* ↑, superoxide anion levels ↑.	(Srisapoome & Areechon, 2017)
Bacillus spp. (ANSCI9, BFAR9, RM3, and RM10)	10^8 CFU g^{-1} of feed.	30 days	Average body weight ↑, absolute growth ↑, specific growth rate ↑, relative growth rate ↑, FCR ↓, disease resistance ↑.	(Samson et al., 2020)
Bacillus spp. (RM10 and BFAR9)	10^8 CFU g^{-1} of feed.	56 days	average body weight ↑, specific growth rate ↑, FCR ↓, condition factor (k) ↑,	(Samson, 2022)
Bacillus spp. Mixture (*Bacillus subtilis* TISTR001, *Bacillus megaterium* TISTR067, and *Bacillus licheniformis* DF001)	(1 × 10^6 CFU/g)	120 days	Immune parameters ↑, *IL*-1β and *TNF*-α gene expressions ↑,	(Doan et al., 2023)
Saccharomyces cerevisiae + *Lactobacillus Acidophilus*	10^8cfu/ml	12 weeks	Final weight ↑, body length ↑, specific growth rate ↑, weight gain ↑, feed intake ↑, FCR ↓, protein efficiency ratio ↑, RBCs ↑, Hb ↑, mortality rate ↓.	(Hassanien et al., 2017)

Saccharomyces cerevisiae + *Bacillus* spp. (*B. subtilis*, *B. megaterium*, and *B. licheniformis*)	5.6×10^8 cfu g^{-1} and 1×10^6 cfu g^{-1}	90 days	Final body weight ↑, weight gain ↑, average daily growth gain↑, aspartate aminotransferase (AST) ↓, alanine aminotransferase (ALT) levels ↓.	(Sutthi et al., 2018)

1.1.5 Synbiotics

Synbiotics, a nutritional supplement, synergistically combine probiotics and prebiotics, exerting beneficial effects on the host animal by positively influencing its intestinal equilibrium, overall health, and growth (Okey et al., 2018). Representing a balance combination of probiotics and prebiotics, synbiotics possess the capability to expedite advancements in aquaculture production. This is achieved through heightened fish growth, enhanced immune system functionality, improved processes of digestion and absorption, efficient disease management, and effective control of water quality (Nguyen et al., 2019; Waagbø & Remø, 2020).

Synbiotics, a combination of probiotics and prebiotics, have a profound impact on organisms by not only enhancing their overall well-being but also by facilitating the implantation of live microbial dietary supplements in the digestive tract, thereby optimizing the gut environment and supporting the fish's health. This is achieved through the selective stimulation of the growth and metabolism of a limited number of health-promoting microorganisms, ultimately contributing to the overall welfare of the organism (Cerezuela et al., 2011; Das et al., 2017). The utilization of synbiotics in aquaculture demonstrates a remarkable synergistic effect on enhancing growth performance, immune response, and disease resistance in aquatic organisms, as evidenced by various studies (Dawood et al., 2018).

The term "synbiotic" specifically refers to a combination of probiotic bacteria and a growth substrate, typically a prebiotic, where the synergistic interaction between the two components results in a distinct impact on growth, immunity, disease prevention, or other parameters that is not achievable by the individual components alone. Prebiotics such as inulin can act as substrates for probiotics in terms of a single or multi species. They are introduced to Nile tilapia as feed additives to secrete supplementary nutrients and enhance growth. Synbiotics also modulate the gut microbiota, promote rebiosis to maintain gut equilibrium, and improve gut function. Synbiotics can stimulate mucosal immunity, promote cell growth and differentiation, and express genes coding for digestive enzymes like amylase, protease, and lipase. This leads to improved digestibility, nutrient utilization, and nutrient absorption. Synbiotics can also improve water quality by promoting beneficial bacteria and inhibiting pathogenic bacteria, as well as enhancing nutrient cycling and increasing resistance to disease (Figure 1. 8).

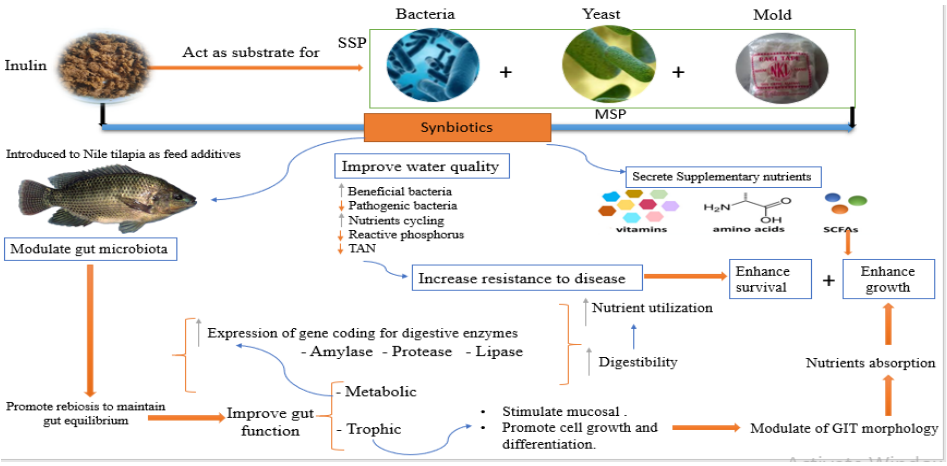


Figure 1. 8 Activities and immunomodulatory mechanisms in Nile tilapia through supplementation with synbiotics
Note: SSP: Single strain probiotics, MSP: Multi species probiotics

1.1.5.1 Selection of prebiotics and probiotics for synbiotics formulations in aquaculture

Since their initial introduction to aquaculture, research has consistently shown that both individual applications of prebiotics and probiotics, as well as their combined use in synbiotics, have a positive impact on the health of aquatic animals. These findings suggest that these dietary supplements can contribute to improved growth, immune function, and disease resistance in aquatic organisms (Yeh et al., 2014). Despite the initial introduction of prebiotics and probiotics in aquaculture, most studies have consistently demonstrated synergistic effects when these components are combined, indicating that the interaction between them produces a more significant and beneficial impact on aquatic animal health compared to their individual use (Zhang et al., 2015). The effects of synbiotics are now considered crucial due to their synergistic interactions, whereas the primary function of prebiotics is to enhance the survival and implantation of probiotics. Developing an effective synbiotic requires evaluating the in vitro ability of prebiotics to selectively stimulate specific probiotics. This process involves ensuring that probiotic bacteria can utilize prebiotics as a carbon source, thereby achieving substantial growth rates and cell yields during fermentation. Nevertheless, this approach is constrained by the limited understanding of the interactions between selective prebiotics and the native microbiota (Kolida & Gibson, 2011). The suitability of specific prebiotic carbohydrates for the selective growth of specific probiotic species is unclear, which is a crucial consideration in the development of synbiotics for aquaculture.

Probiotics can metabolize certain prebiotic oligosaccharides, enabling their selective proliferation within the intestinal tract. This metabolism results in the production of primary and secondary metabolites that confer health benefits to the host. To create potent synbiotic formulations, it is essential to assess and fine-tune the combinations of probiotics and prebiotics based on their prebiotic activity, which indicates the substrate's effectiveness in supporting probiotic growth, as reported by Mazzola et al. (2015). The complementary effect in synbiotics involves selecting a probiotic based on its specific effects on the host, and then choosing a prebiotic that selectively enhances the growth and activity of the probiotic. The prebiotic does not directly interact with the probiotic but rather supports its growth and activity within its target range, enhancing the overall benefits of the synbiotic (Figure 1.9).

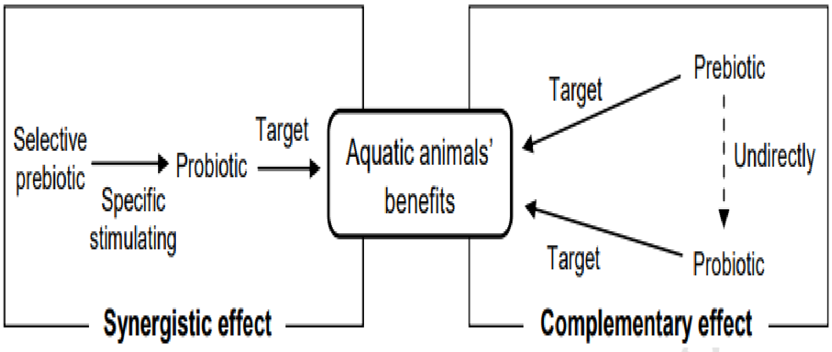


Figure 1. 9 Synbiotic approaches in aquaculture
Source: (Huynh et al., 2017).

1.1.5.2 Application of synbiotics in tilapia farming

The use of synbiotics in tilapia farming has gained significant attention in recent years due to its practicality and numerous benefits for the aquaculture industry (Sewaka et al., 2018). Research has extensively investigated the sources and effects of synbiotics on various aspects of tilapia farming, including growth rates, feed efficiency, digestive enzyme function, intestinal morphology, blood parameters, immune response, disease resistance, and survival rates. (Ismail et al., 2019; Doan et al., 2019).

Synbiotics have been recognized as crucial growth and immunity-enhancing factors in both freshwater and marine fish, thereby expanding their potential benefits to a broad spectrum of aquatic species (Rohani et al., 2022). By introducing synbiotics into the diet, the favorable bacteria in the intestinal tract can thrive, enhancing overall intestinal enzyme activity. This heightened enzymatic activity increases feed digestibility, ultimately leading to improved growth performance in the host species (Ahmadnia et al., 2012). Moreover, exogenous enzymes complement the action of endogenous enzymes, extending the digestion process and enabling more complete hydrolysis of complex substances. The addition of supplemental synbiotics also triggers an increase in the secretion of digestive enzymes, thereby facilitating the breakdown of complex molecules into simpler ones. This process ensures the availability of essential nutrients crucial for growth (Dehaghani et al., 2015). Enhanced enzyme activities bolster the host fish species' capacity to efficiently degrade nutrients, resulting in improved digestion rates and feed utilization (Cerezuela & Meseguer, 2011; Rohani et al., 2022).

Extensive studies have focused on the impact of synbiotics on Nile tilapia growth performance, consistently demonstrating positive outcomes. The application of synbiotics has been linked to enhanced growth rates, improved innate immunity, and increased resistance to bacterial infections, highlighting their potential in improving the overall health and well-being of Nile tilapia (Hassaan et al., 2015; Sirbu et al., 2022). Notably, synbiotics have been shown to mitigate the adverse effects on the growth performance, blood health, and immune response of Nile tilapia reared under low-temperature conditions, making them a valuable tool for enhancing the resilience of this economically significant fish species (Gewaily et al., 2021). The application of synbiotics in Nile tilapia farming has been a significant area of research since 2010. The use of probiotics and prebiotics as feed additives has been explored to improve growth performance, physiological conditions, and disease resistance in Nile tilapia. This approach involves combining probiotics with prebiotics to enhance the beneficial effects on the fish, leading to better overall health and well-being (Figure 1. 7).

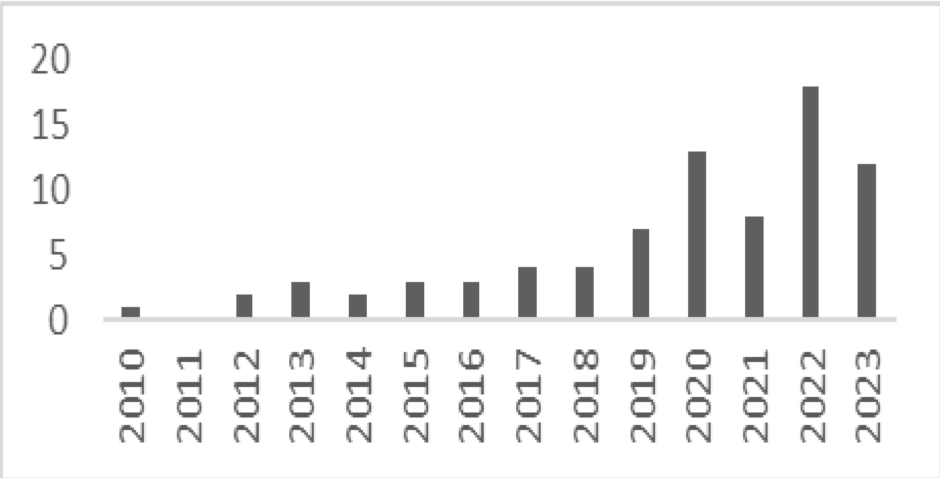


Figure 1. 7. Application of synbiotics in Nile tilapia farming 2010 – 2023

Research has consistently demonstrated that dietary supplementation with probiotics, prebiotics, and beta-glucans can have substantial positive effects on the growth, immune response, oxidative status, and overall health of Nile tilapia and other fish species. These supplements have been found to enhance growth performance, improve body protein content, increase digestive enzyme activity, and boost immune and oxidative responses. Furthermore, studies suggest that these dietary additives may also influence the balance of intestinal microbiota, improve intestinal morphology, and protect against bacterial infections, highlighting their potential in promoting the overall well-being of fish (Table 1. 6). Overall, the evidence supports the potential of using synbiotics, probiotics, and prebiotics as efficient and sustainable approaches for enhancing fish health and production (Sewaka et al., 2018; Dawood et al., 2019; Doan et al., 2019; El-Nobi et al., 2021; Mohammadi et al., 2021; Fath El-Bab et al., 2022; Hersi et al., 2023).

Table 1. 6 Summary of synbiotic applications in Nile tilapia farming

Synbiotics	**Dosage**	**Duration**	**Response**	**Reference**

Aspergillus oryzae (ASP) and β-glucan (BG)	1 ASP + 1 BG g.kg^{-1}	60 days	FBW ↑, WG ↑, SGR↑, FER ↑, PER ↑, activity of antioxidative enzymes (SOD and CAT) ↑, oxidative enzyme (MDA)↓, NBT↑, IgM↑, lysozyme↑, bactericidal ↑, phagocytosis ↑.	Dawood et al., 2019)
β-glucan and *Bacillus coagulans*	0.1 g BG + 1 g *B. coagulans* kg−1; 0.1 g BG + 2 g *B. coagulans* kg−1.	14 weeks	Growth performance ↑, feed efficiency parameters ↑, body Composition ↑, antioxidant activity ↑, anterior intestine villus ↑, *HSP70* and *IL-1*β gene expression ↓, *IL-8* and *GH* geneExpression ↑.	Fath El-Bab et al., 2022
Jerusalem artichoke (JA) and *Lactobacillus rhamnosus*	10 g kg^{-1} JA+10^{8} CFU g^{-1} L. *rhamnosus*	30 days	SGR ↑, ADG↑, FCR ↓, glucose ↑, total protein ↑, total cholesterol levels ↑, lysozyme activity ↑.	Sewaka et al., 2018
Saccharomyces cerevisiae, β-glucan, and MOS	1 × 1011 cfu/L, 25 g/L, 35 g/L	8 weeks	Total protein↑, globulin↑, albumin↑, nitric oxide ↑, lysozyme activity ↑, serum glucose ↑, cholesterol ↑, triglycerides ↑, catalase ↑, superoxide dismutase ↑, glutathione peroxidase ↑, cumulative mortality rate ↓.	El-Nobi et al., 2021
MOS+ *Lactobacillus Plantarum, Saccharomyces boulardii, and Lactobacillus acidophilus*	2g kg^{-1}+ 1.5 × 10^{9} cob g^{-1} + 5 × 10^{9} cob g $^{-1}$+ 5 × 10^{9} cob g^{-1}	85 days	Gut microbiota ↑, body composition ↑, tissue histomorphology ↑.	Hersi et al., 2023
PHDP + *Pediococcus acidilactici (PA)*	PHDP (0.1%) + PA (0.2%)	56 days	SGR ↑, digestive enzymes activity ↑, immune response ↑, resistance against Aeromonas *hydrophila* ↑.	Mohammadi et al., 2021
CDXOS + *Lactobacillus plantarum* CR1T5 (LP)	10 g kg^{-1} CDXOS +10^{8} CFU g^{-1} *L. plantarum* CR1T5	12 weeks	FBW ↑, WG ↑, SGR ↑, FCR ↓, lysozyme and peroxidase activities ↑, resistance against *Streptococcusagalactiae*	Doan et al., 2019
Lactobacillus acidophilus + fructooligosaccharides+ mannan oligosaccharides	84×10^{7}CFU g^{-1}	84 days	FBW ↑, FCR ↑, PER ↑, body chemical composition ↑, hematological parameters ↑, insulin-like growth factor ↑, expression of *GH* and *GHR1*	Hassaan et al., 2015
Betaplus® + Technomos®	BetaPlus® probiotics and TechnoMos® prebiotics in a ratio of 1:1%	50 days	Growth performance ↑, health profiles ↑, resistance to infection with *Aeromonas hydrophila* and *Pseudomonas fluorescens* ↑, survival rate ↑	Sîrbu et al., 2022

Note: FBW: Final body weight, WG: weight gain, SGR: specific growth rate, FER: Final energy retention, PER: Protein efficiency ratio, CDXOS: corncob derived xylooligosaccharides, FCR: feed conversion ratio

1.2 Statement of problems

The high cost of fish feed ingredients and frequent disease outbreaks hinder the full potential of aquaculture's intensification and commercialization. Researchers have increasingly focused on developing feed additives, particularly prebiotics, probiotics, and their combination (synbiotics) to enhance feed efficiency in aquaculture. However, there is insufficient information on the in-vitro evaluation of synbiotics combination, which is an important topic to investigate before introducing a synbiotics mixture into in-vivo experiments. Therefore, there is a need to conduct an in-vitro efficacy test to elucidate the specificity of the prebiotic for selective stimulation of the chosen probiotic microbes. The aims of this research are to isolate and characterize inulin from sweet potatoes then, to determine the optimal synbiotic combinations between the isolated inulin and multi-species probiotics (*Bacillus* spp., *Rhizopus* sp., and *Saccharomyces* sp.) through in-vitro evaluations based on microbial growth and digestive enzyme activities. Finally, to introduce the best combinations of synbiotic to *O. niloticus* feed based on growth parameters. The supplementation of synbiotics is expected to improve the growth performance, body weight composition, digestive enzymes activity, and histological parameters of Nile tilapia (*O. niloticus*).

Based on the background above, the problems statement can be made as follow:

1. How can inulin be effectively extracted and characterized from sweet potatoes (*Ipomoea batatas* L.) using different evaporation techniques?
2. What are the synergistic effects of inulin and multi-species probiotics (*Bacillus* spp., *Saccharomyces* sp., and *Rhizopus* sp.) on microbial growth and digestive enzyme activity using an in vitro method.
3. How do dietary synbiotics (inulin and multi-species probiotics) affect the growth performance, feed digestibility, enzymes activity, chemical body composition, and liver histology of *O. niloticus*?

1.3 General objectives

1. To investigate and develop a method for the evaporation and characterization of inulin from sweet potatoes (*Ipomoea batatas* L.) using different evaporation techniques.

2. To evaluate the synergistic effects of inulin and multi-species probiotics (*Bacillus* spp., *Saccharomyces* sp., and *Rhizopus* sp.) using in-vitro methods based on microbial growth and digestive enzymes activity.
3. To assess the effect of dietary synbiotics supplementation on growth performance, feed digestibility, enzymes activity, chemical body composition, and liver histology of *O. niloticus*.

1.4 Research hypothesis

H_1: The use of different evaporation techniques will significantly affect the characterization of inulin extracted from sweet potatoes (*Ipomoea batatas* L.)
H_2: The invitro evaluation of synbiotics combination between extracted inulin and multi-species probiotics (*Bacillus* spp*, Saccharomyces* sp*, and Rhizopus* sp*)* will significantly enhance microbial growth and digestive enzyme activity.
H_3: Dietary supplementation of synbiotics will significantly improve the growth performance, feed digestibility, enzyme activity, chemical body composition, and liver histology of *O. niloticus*.

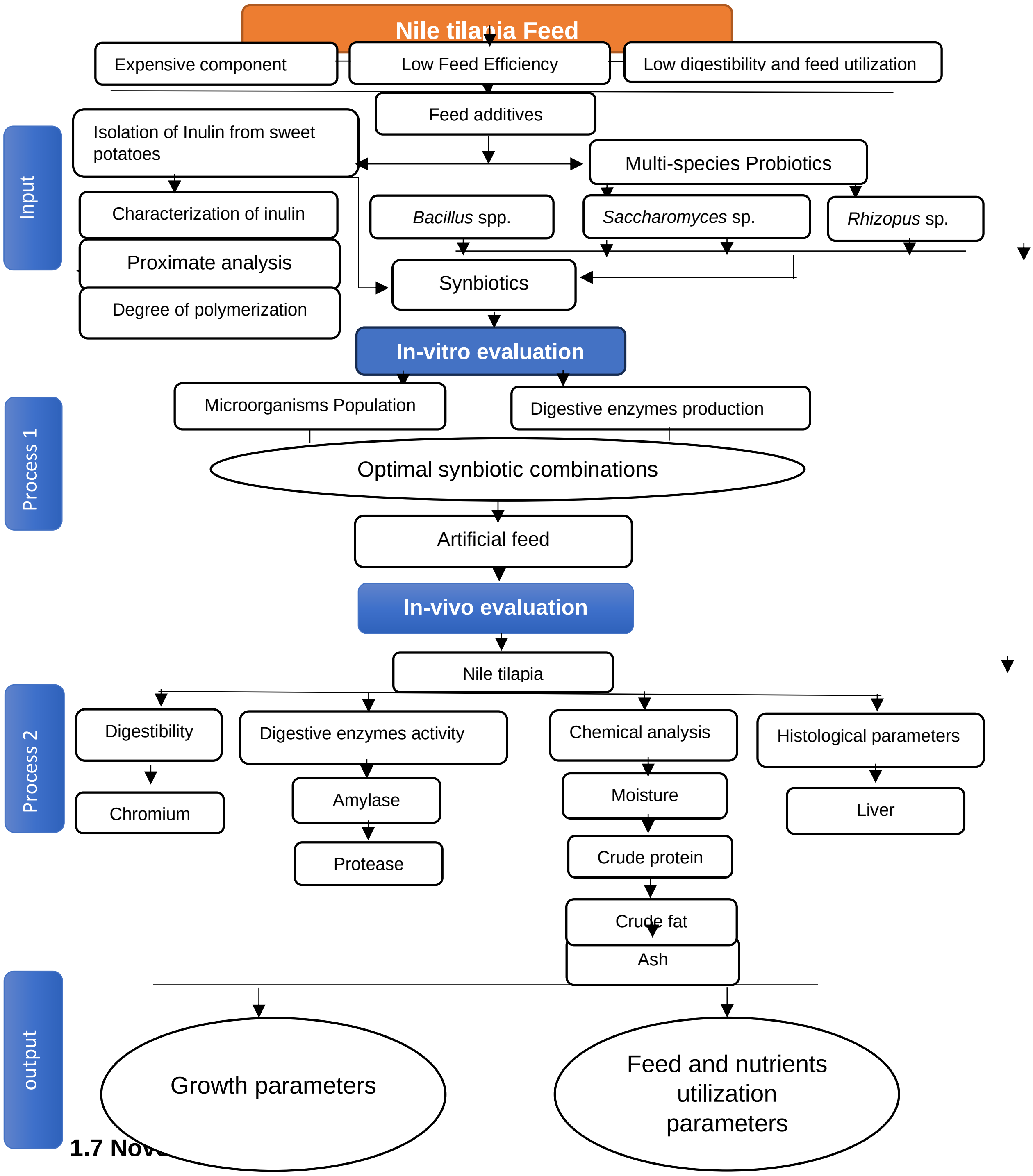
1.5 Research benefits

The aquaculture sector faces significant challenges, including high feed costs, low digestibility, and inefficient feed utilization. With the recent ban on antibiotics and therapeutics due to environmental and consumer health concerns, there is an urgent need for sustainable alternatives to improve feed efficiency and reduce costs. The use of prebiotics, probiotics, and their combination (synbiotics) offers a promising solution. This strategy is environmentally friendly and has the potential to enhance feed utilization, growth performance, and overall health of fish.

The addition of inulin, a prebiotic extracted from sweet potatoes, combined with multi-species probiotics, is expected to enhance the fish's gut microbiota, promoting the activity of beneficial enzymes such as amylase and protease. These enzymes can improve nutrient digestion, increase feed efficiency, and potentially reduce the cost of aquaculture feeds.

The results of the study would be very important for fish specialists and the outcomes of this study are crucial for aquaculture professionals, offering potential solutions to reduce feed costs, improve digestibility, and enhance feed conversion efficiency. Furthermore, the findings will contribute valuable knowledge to the fields of fish aquaculture and nutrition, serving as a reference for future research on sustainable farming methods in aquaculture.

1.6 Research framework



1.7 Nov

This study presents three significant novelties in the extraction and application of inulin from sweet potatoes. First, it establishes that the evaporation by using freeze-drying method is better than rotary evaporator, resulted higher total and reducing sugar content while improving nitrogen-free extract levels and classifying the extracted inulin as short-chain prebiotics (DP 1-9) with fast metabolization potential. Second, for the first time, this study evaluates the synergistic effects of inulin extracted from sweet potatoes combined with multi-species probiotics (*Bacillus* spp., *Rhizopus* sp., and *Saccharomyces* sp.) in vitro, demonstrating potential benefits for enhanced microbial growth and digestive enzymes activity. Third, it is the first study to apply this synbiotics combination in Nile tilapia farming, revealing significant enhancements in growth performance, feed digestibility, enzyme activity, chemical body composition, and liver histology, offering a sustainable and innovative dietary strategy to advance fish growth and aquaculture productivity.

CHAPTER II

EXTRACTION AND CHARACTERIZATION OF INULIN FROM SWEET POTATOES (Ipomoea batatas L.)

2.1 Abstract

Inulin a bioactive carbohydrate found in sweet potatoes, has gained considerable attention due to its prebiotic properties and potential applications in the food and pharmaceutical industries. This study aimed to optimize the extraction and characterization of inulin from sweet potatoes (*Ipomoea batatas* L.) using two evaporation methods: a rotary evaporator and a freeze-dryer. The isolated inulin was characterized through Fourier Transform Infrared Spectroscopy (FT-IR) and proximate analysis. The results demonstrated that the freeze-dryer method yielded higher values of total sugar (28.78 ± 0.31 µg/mL) and reducing sugar (16.55 ± 1.77 µg/mL) compared to the rotary evaporator method (23.55 ± 1.63 µg/mL and 10.85 ± 0.49 µg/mL, respectively. Additionally, the freeze-dryer method produced lower levels of water (42.64 ± 0.62%), crude fat (1.33 ± 0.31%), and crude protein (7.21 ± 0.87%) content but a higher nitrogen-free extract content (43.16 ± 1.78%) when compared to the rotary evaporator method (47.01 ± 1.40, 3.71 ± 0.85, and 8.01 ± 1.27 %, respectively). FT-IR spectra confirmed the presence of inulin in both samples, with distinct absorption bands characteristic of the inulin structure, particularly the OH stretch between 3000–3500 cm-1. Commercial inulin exhibited a peak at 3381 cm^{-1}, while the evaporator-derived inulin showed a peak at 3258 cm^{-1}, and the freeze-dryer-derived inulin exhibited a peak at 3271.95 cm^{-1}. Overall, these findings highlight the freeze-dryer method as the more efficient and effective technique for extraction inulin from sweet potatoes, offering higher yield and purity, which is crucial for potential applications

Keywords: Evaporator, freeze dryer, FT-IR spectroscopy, inulin, sweet potatoes.

2.2 Introduction

Sweet potato (*Ipomoea batatas* L.) has become a research focus in recent decades because of its nutritional and functional properties, easily available and economical compared to other natural materials. Its leaves, stems and roots are a valuable source of bioactive carbohydrates, lipids, proteins, carotenoids, anthocyanins, phenolic acids and flavonoids. These bioactive metabolites possess many biological activities, such as antioxidant, antidiabetic, anticancer, hepatoprotective, antimicrobial, antiulcer and immunostimulant activities (Alam, 2021). Notably, inulin is one such bioactive compound found in sweet potatoes (Hiel et al., 2019).

Inulin is a linear biopolymer made up of D-fructose units connected by (2-1) glycosidic bonds. Typically, a glucose molecule resides at the terminal end of each fructose chain and is connected by a -1,2 bond, as in sucrose (Yu et al., 2024). Inulin exhibits a range of properties, operating as thickeners and fat substitutes in low calorie products and prebiotic activity, which are of particular interest for the food industry. Currently, inulin type fructans are the most popular and extensively studied prebiotics (Jackson et al., 2023).

The monomeric unit count and relative molar mass of inulin are mostly influenced by the plant species utilized throughout the isolation process (Akram & Garud, 2020). The range of inulin polymerization, as measured by its degree of polymerization (DP), spans from 1 to 70. Inulin with a DP of less than 10 is classified as oligofructose or shorter-chain inulin, while inulin with a DP greater than 23 is referred to as poly-fructose or longer-chain inulin (Liu et al., 2016; Beccard et al., 2019).

The extraction and characterization of inulin from sweet potatoes have attracted significant interest in recent years, driven by the growing demand for natural and functional ingredients in the food and pharmaceutical industries. Inulin possesses several physicochemical properties that make it an appealing ingredient for various applications, such as its solubility in water, ability to form gels, and resistance to digestion by human enzymes (Yudhistira et al., 2020). These properties contribute to its potential use as a dietary fiber supplement, fat replacer, and stabilizer in food formulations, as well as its role in promoting gut health and modulating the immune system.

The extraction of inulin from sweet potatoes involves a series of steps, including the selection and preparation of raw materials, extraction techniques, purification processes, and characterization methods (Alexander et al., 2023). Various extraction methods, such as hot water extraction, enzymatic hydrolysis, and ethanol precipitation, have been employed to isolate inulin from the tubers. Furthermore, purification techniques like filtration, centrifugation, and chromatography are applied to obtain a high-quality inulin extract with desirable properties and purity (Yudhistira et al., 2022).

To evaluate the quality, functionality, and potential applications of inulin extracted from sweet potatoes, its chemical composition, molecular weight distribution, degree of polymerization, and functional properties need to be characterized. Analytical methodologies, including high performance liquid chromatography (HPLC), gas chromatography (GC), infrared spectroscopy (IR), and nuclear magnetic resonance (NMR) spectroscopy, are frequently utilized for the purpose of elucidating the structural and physicochemical properties of inulin (Petkova & Denev, 2015; Cortés-Herrera et al., 2019; Retnaningtyas et al., 2022). Understanding these properties is essential for assessing the quality, functionality, and potential applications of inulin extracted from sweet potatoes.

The extraction and characterization of inulin from sweet potatoes have received limited attention in previous research. Until now, commercial inulin is predominately extracted from chicory root due to its high inulin content (14.9–18.3 %) and high tolerance to cold weather (Redondo-Cuenca et al., 2021). This study aims to investigate the optimal conditions for extracting inulin from sweet potatoes (*Ipomea batatas* L.) and to characterize it chemical properties, thereby contributing to the development of inulin as prebiotics. The extraction and characterization of inulin from sweet potatoes holds significant promise for the development of natural and functional ingredients.

2.2.1 Objectives

This study aims:

1. To optimize the extraction process for isolating and characterizing inulin from sweet potatoes (*Ipomoea batatas* L.) using different evaporation techniques.
2. To assess the impact of various evaporation techniques on the total sugar content, reducing sugar, and degree of polymerization of the isolated inulin.
3. To compare the proximate composition of isolated inulin through different evaporation techniques.

2.3 Materials and Methods

2.3.1 Place and time of the research

Sweet potatoes used in this study were obtained from local markets in Makassar City, South Sulawesi, Indonesia. Furthermore, isolation of inulin was conducted at the Laboratory of Fish Parasites and Diseases, Faculty of Marine Sciences and Fisheries, Hasanuddin University, Makassar, Indonesia from May to September 2023. Commercial inulin was obtained from Yasma Natura, Sidoarjo, East Java, Indonesia.

2.3.2 Preparation of inulin extract

The process of sample preparation started with washing, peeling, and chopping of sweet potato with a medium size (1-2 mm), followed by drying in an oven at a temperature of 50ºC for 48 hours. The dried sweet potatoes were then ground into flour and sieved using an 80 mesh sieve. The process of sample preparation was based on the method proposed by Kosasih et al. (2015), with some modifications.

The results of the extraction preparation were utilized for the extraction process of sweet potatoes (sweet potato flour). The process of sweet potatoes extraction included mixing the flour and aquadest (1: 5) then was soaked for 1 hour, heating in water bath by stirring the solution at 80°C for 30 minutes, the solution was filtered using double filter paper, this process was carried out twice so that all inulin could be extracted (Kosasih et al., 2015).

2.3.3 Solvent evaporation

The solvents were evaporated using two techniques. First, vacuum rotary evaporator at a temperature of 73°C, with a speed of 60 rpm for 30 minutes. Second, a freeze-dryer.

2.3.4 Precipitation process of inulin

Inulin was precipitated by adding 95% ethanol in a 1:2 ratio, homogenized, and then soaked for 12 hours at room temperature. Furthermore, separated by a centrifuge for 15 minutes (5000 rpm) and dried in an oven at 60°C for 6 hours (Yudhistira et al., 2020).

2.3.5 Analysis of total sugar, sugar reduction, degree of polymerization (DP), and concentration of isolated inulin

The determination of total sugar content in 1% inulin was conducted using the phenol-sulfuric acid method as described by Yanti et al. (2019). Fructose solutions with concentrations ranging from 200 to 800 µg/mL were used as the standard. 1mL of the sample was transferred into a test tube and add 0.5 mL of 5% phenol. The mixture was shaken, and 2.5 mL of H2SO4 was perpendicularly poured into the test tube. After standing for 10 minutes, the solution was shaken and placed in a water bath for 15 minutes before cooling to room temperature. Subsequently, the solution was diluted with 8 mL distilled water and thoroughly homogenized. The final step included measuring the absorption of orange-yellowish color at a wavelength of 350 nm. For the determination of sugar reduction, the DNS method was used following Miller (1959) method. Fructose solutions with concentrations ranging from 200 to 600 µg/mL were used as the standard. 75 µL of 1% inulin and 75 µL of dinitro salicylic acid (DNS) reagent was transferred into a microtube. Further, the mixture was placed in a boiling water bath for 10 min and cooled to room temperature. The mixture was diluted with distilled water and homogenized. Average inulin degree of polymerization as calculated based on the total sugar content per reducing sugar content using the formula of Saengkanuk et al. (2011).

2.3.6 Proximate Chemical Analysis

The proximate analysis of isolated inulin was conducted following the standard methods by the Association of Official Analytical Chemists (AOAC, 2019). The analysis included the determination of moisture, crude protein (CP), crude fat (CF), crude fiber, ash, and nitrogen-free extract (NFE). The Kjeldahl method was employed to determine the protein content by converting the nitrogen content into a protein percentage using a conversion factor of 6.25. The fat content was measured using the Soxhlet method, and ash content was obtained by heating samples in a muffle furnace until a constant weight was achieved. The NFE was calculated by subtracting the sum of ether extract, crude protein, crude fiber, and ash percentages from 100% of the dry matter.

2.3.7 Characterization of inulin

The study used a fourier transform infrared spectrometer (FT-IR) to compare and characterize the structural features of functional groups in both commercial and isolated inulin (Figure 2.1). Approximately three mg of inulin samples were mixed with around 200 mg of potassium bromide anhydrous (KBr) and ground into a fine powder using a pestle and mortar. The FT-IR spectra were then analyzed to identify the existing functional groups and to compare them, as documented by previous studies (Fares & Salem 2015; Nurdila et al., 2019). FT-IR spectra of the samples were obtained using an FT-IR spectrophotometer (FT-IR 8400S, Shimadzu, Japan) with a resolution of 4 cm^{-1} and a test speed of 1 cm/s, within the range of 400–4000 cm^{-1}.

Figure 2. 1 Isolated inulin from sweet potatoes

2.3.8 Statistical analysis

The analysis of isolated inulin included measurements of total sugar, sugar reduction, degree of polymerization, and proximate analysis, all of which were analyzed descriptively. The results were presented as a means with standard deviations (SD) (mean ±SD).

2.3 Results and Discussion

2.4.1 Results

2.4.1.1 Analysis of total sugar, sugar reduction, and degree of polymerization

The results of total sugar and sugar reduction, and degree of polymerization of isolated inulin are presented in (Table 2.1). The freeze-dryer method resulted in higher values for both total sugar and sugar reduction compared to the evaporator method. The total sugar content for the freeze-dryer was (28.78 ± 0.31), while it was (23.55 ± 1.63) for the evaporator method. Similarly, the sugar reduction for the freeze dryer method was (16.55 ± 1.77), whereas it was (10.85 ± 0.49) for the evaporator method. Additionally, the degree of polymerization was lower for the freeze dryer method (1.73 ± 0.42) compared to the evaporator method (2.17 ± 0.09). Inulin concentration also was higher in freeze dryer method (8.75) compared to the evaporator method (6.88%).

Table 2.1 Total sugar, sugar reduction, degree of polymerization, and concentration of isolated inulin (Mean ± SD).

Method	Parameters			
	Total sugar (µg/mL)	Sugar reduction (µg/mL)	Degree of polymerization	Inulin Concentration %
Evaporator	23.55 ± 1.63	10.85 ± 0.49	2.17 ± 0.09	6.88
Freeze dryer	28.78 ± 0.31	16.55 ± 1.77	1.73 ± 0.42	8.75

2.4.1.2 Proximate analysis of isolated inulin

The proximate analysis of isolated inulin presented in (Table 2.2). The proximate analysis of isolated inulin reveals differences between drying methods. The evaporator method retains more water (47.01%) compared to freeze-drying (42.64%). Crude protein is slightly higher in the evaporator sample (8.01%) than in the freeze-dried sample (7.21%), while crude fat content is notably greater in the evaporator (3.71%) versus freeze-drying (1.33%). Both methods report no crude fiber, indicating low fiber content in isolated inulin. The nitrogen-free extract (NFE) is higher in the freeze-dried sample

(43.16%) compared to the evaporator (35.53%), suggesting enhanced digestibility and sweetness. Ash content remains similar across both methods, at 5.73% for evaporator and 5.67% for freeze-dryer.

Table 2. 2 Proximate analysis of isolated inulin (Mean ± SD).

Method	Proximate Composition %					
	Water	Crude protein	Crude fat	Crude fiber	NFE*	Ash
Evaporator	47.01 ± 1.40	8.01 ± 1.27	3.71 ± 0.85	0.00 ± 0.00	35.53 ± 2.02	5.73 ± 1.67
Freeze -dryer	42.64 ± 0.62	7.21 ± 0.87	1.33 ± 0.31	0.00 ± 0.00	43.16 ± 1.78	5.67 ± 0.49

*NFE: Nitrogen-free extract

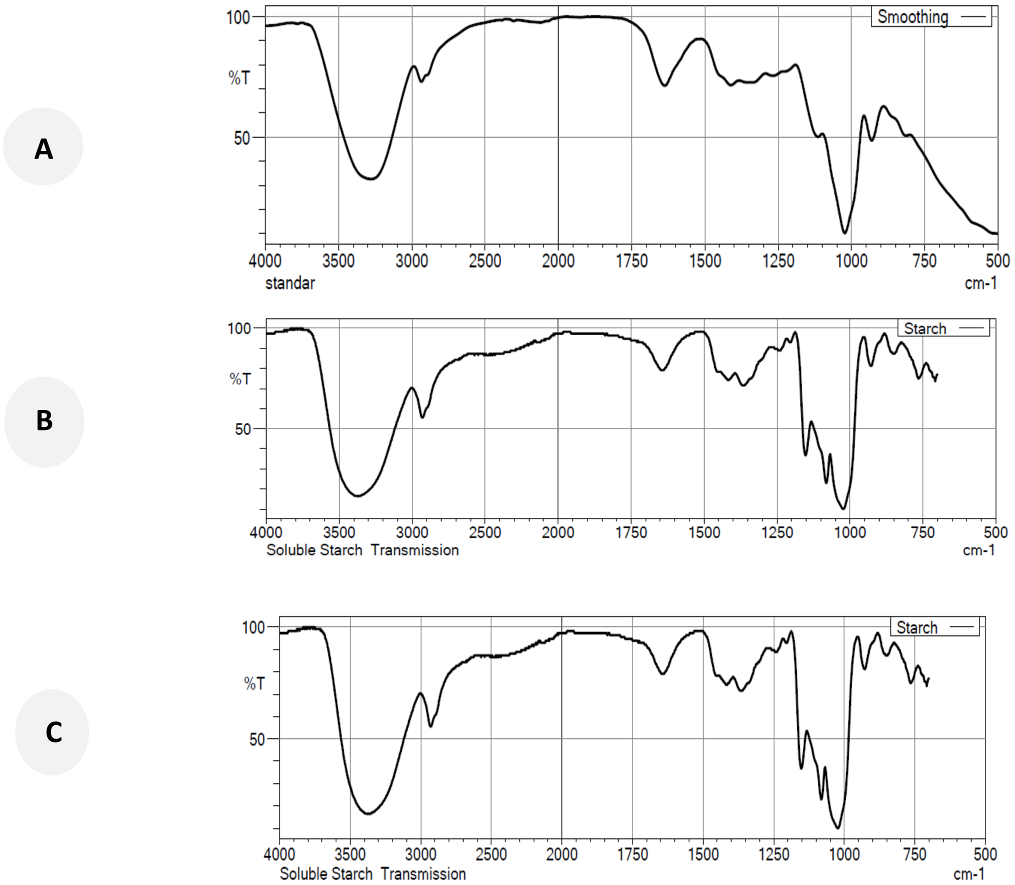
2.4.1.3 Characterization of inulin

The results of FT-IR analysis showed a strong asymmetric stretching of -OH bonds at a band around 3.000 – 3.500 cm^{-1}, with commercial inulin exhibiting a peak at 3.381 cm^{-1}, evaporator at 3258 cm^{-1}, and freeze dryer at 3.271.95 cm^{-1} (Figure 2.1). This characteristic absorption peak is a clear indication of the presence of inulin in both samples. Moreover, the bands in the range of 2.800-2.950 cm^{-1} exhibited medium intensity and corresponded to the asymmetric and symmetric stretching of C-H bonds in the CH2 and CH3 groups.

The isolated inulin showed an increased low-intensity band in the range of 1.590-1.650 cm^{-1}, which can be attributed to the presence of C=C bonds. Two bands at 1.000 and 1.100 cm^{-1} were present in both spectra, which are associated with in-plane bending vibrations and internal deformations of CH, CH_2, and OH groups from the fructose ring. Additionally, the peak observed in the range of 900-950 cm^{-1} was more prominent in the isolated inulin spectra. This region is dominated by various stretching and deformation vibrations related to C-C, C-O stretching, C-O-H, and C-O-C deformations found in oligo- and polysaccharides.

Table 2. 3 Interpretation of FT- IR spectra of commercial and isolated inulin

	Wave number cm^{-1}	IR bands cm^{-1}	Interpretation
Commercial inulin	3100-3400	3381	OH
	2800-2950	2930.11	vC–H as (CH2)
	1590-1650	1630.95	Absorption of water
	1000 - 1100	1014.77	vC–O (C–O) C–O
	900-950	930.74	vC–O (C–O)
Evaporator	3100 - 3400	3258	OH
	2800-2850	2935.14	v C–H as (CH2)
	1590-1650	1636.70	Absorption of water
	1000-1100	1022.67	vC–O (C–O) C–O
	900-950	930.74	vC–O (C–O)
Freeze dryer	3100 - 3400	3271.95	OH
	2800-2950	2931.54	v C–H as (CH2)
	1590-1650	1596.48	Absorption of water
	1000-1100	1023.38	vC–O (C–O) C–O
	900-950	920.69	vC–O (C–O)



A: Commercial inulin, B: Freeze-dryer, C: Evaporator

Figure 2. 2 FT–IR spectra of commercial and isolated inulin from sweet potatoes

2.4.2 Discussion

Inulin, naturally found in sweet potatoes, has garnered significant attention for its potential health benefits and versatility in various industrial applications. The isolation and characterization of inulin from sweet potatoes are crucial steps in understanding its properties and optimizing its extraction methods. This study aims to investigate the optimal conditions for extracting inulin from sweet potatoes (*Ipomea batatas* L.) and to characterize it and chemical properties, thereby contributing to the development of inulin as prebiotics.

The analysis of total sugar and sugar reduction revealed that the evaporation by freeze-dryer produced inulin with higher total sugar content and sugar reduction compared to the evaporator. This indicates that the freeze-drying process preserves the sugar content present in the isolated inulin, while also promoting sugar reduction. The discrepancy in total sugar content and sugar reduction between the freeze dryer and evaporator methods can be attributed to the preservation capabilities of freeze-drying in maintaining sugar levels in isolated inulin, as well as promoting sugar reduction (Yue et al., 2022). Freeze-drying is known for its effectiveness in preserving sensitive biological materials by utilizing low temperatures and pressures, which could similarly benefit the sugar content in inulin (Assohoun, 2022). Additionally, the selection of excipients in the freeze-drying process plays a crucial role in enhancing the shelf life and stability of the final product, potentially influencing the sugar content and reduction levels in the inulin (Alfano et al., 2022). Therefore, the superior performance of the freeze-dryer method in retaining higher total sugar content and promoting sugar reduction compared to the evaporator method underscores the importance of the drying technique and process parameters in preserving the sugar composition of inulin.

Furthermore, the evaporation by freeze-dryer resulted lower degree of polymerization compared to the evaporator. Degree of polymerization refers to the number of sugar units in the inulin molecule. The statement regarding the preferential metabolism of prebiotic oligosaccharides with a shorter degree of polymerization, suggesting faster utilization by gut microbiota for nourishment (Shiri et al., 2023), aligns with findings on inulin's molecular structure and functionality (Wienberg et al., 2022; Thielemans et al., 2023). Research indicates that inulin with a lower degree of polymerization, achieved through methods like freeze-drying, may result in smaller molecules with improved solubility and functional properties, potentially making them more beneficial for applications in food formulation or as dietary supplements. These smaller inulin molecules could be more readily metabolized by gut microbiota, promoting their growth and activity, thus supporting the notion that inulin with a lower degree of polymerization could be more efficiently utilized by the gut microbiome as a nutrient source, ultimately contributing to enhanced gut health and overall well-being (Mugwanya et al., 2022).

The evaporation by freeze-dryer significantly outperformed the evaporator for removing water from inulin extracted from sweet potatoes, yielding a lower water content that enhances stability and shelf life (Nguyen et al., 2022). Specifically, the freeze-dryer achieved an inulin concentration of 8.75% (w/w) in the dried samples, compared to only 6.88% (w/w) from the evaporator method. This discrepancy emphasizes the critical role of drying methods in accurately determining inulin concentration, as the higher water content in samples processed through evaporation can mask true inulin yields. Additionally, the freeze-dryer produced slightly lower values for crude protein and crude fat, likely due to its gentle drying conditions that help preserve native protein and lipid content. However, further research is necessary to evaluate how these differences in protein and fat content may influence the functional properties of the isolated inulin. Previous studies support that freeze-drying not only yields higher inulin concentrations but also ensures superior quality, making it a preferred choice for inulin extraction from sweet potatoes and similar sources (Savedboworn et al., 2019).

Interestingly, both evaporation techniques yielded negligible crude fiber content in the isolated inulin, indicating that the fiber-rich components of the sweet potatoes were effectively removed during the isolation process. This resulted in a highly purified form of inulin, which is particularly desirable for applications in the food and pharmaceutical industries where purity is crucial. Notably, the freeze-dryer method produced a higher nitrogen-free extract (NFE) value compared to the evaporator method, suggesting a greater concentration of carbohydrates in the isolated inulin. This finding implies that freeze-drying preserves carbohydrate content more effectively, potentially leading to a higher yield of inulin with superior nutritional value. Research highlights the effectiveness of different isolation methods in removing fiber-rich components and enhancing the carbohydrate concentration in the final inulin product (Starovoitov et al., 2023). Furthermore, studies on freeze-dried potatoes have demonstrated that the freeze-drying process effectively preserves carbohydrate content, thereby increasing the nutritional value and potentially improving the yield of inulin, which is essential for applications requiring highly pure inulin (Andrianto et al., 2022). These insights underscore the advantages of freeze-drying in obtaining a high-quality inulin product suitable for various industrial applications.

One study comparing the effects of freeze-drying and foam mat drying on the characteristics of inulin from gembili found that freeze drying is a more effective method for extracting inulin from gembili. The study found that samples dried using foam mat drying contained about 9.38% inulin, while those dried using freeze-drying had a higher inulin content (Indah et al., 2020). Freeze drying is a highly effective method for extracting bioactive compounds from plant material, such as sweet potatoes. This process removes up to 98% of water from samples, significantly more than traditional drying methods that typically remove only 70-80% of water. Freeze-dried products retain more vitamins and nutritional value compared to conventionally dried products (Krakowska-Sieprawska et al. 2022). These findings highlight the potential benefits of using freeze-drying as a method for extracting compounds from samples, including inulin from sweet potatoes.

The FTIR infrared spectrum of sweet potato inulin samples indicate that the absorption numbers and results are very similar to those of commercial inulin. This finding is consistent with previous studies by Akram and Garud (2020) and Melanie et al. (2015), which reported similar bands for inulin. The absorption numbers of the sweet potato samples are within the range of absorption waves of functional groups that indicate the presence of inulin in the sample. According to Melanie et al. (2015), the hydroxyl group (OH) is a group that shows the main characteristics of inulin. This hydroxyl group is in the absorption range between 3550-3230 cm-1 and has a bond band with an asymmetrical shape. Furthermore, the bands observed at 1590-1650 cm-1, 1000-1100 cm−1, 900-950 cm−1, and 1030 cm−1 are characteristic of stretching vibrations of (C–C), (C–O–C), and (C–O) groups, as reported by Grube et al. (2002). These findings provide strong evidence for the presence of inulin in sweet potato samples.

2.4 Conclusion

1. Evaporation by freeze-dryer method yielded higher total sugar, sugar reduction inulin concentration, and lower degree of polymerization compared to the evaporator method.
2. Evaporation by freeze-dryer method resulted in lower water, crude fat, and crude protein content, and higher nitrogen-free extract (NFE) content.
3.The FT-IR spectra confirmed the presence of inulin in both methods, with characteristic peaks for -OH, C-H, and fructose ring structures.