The Physiochemical Characteristics, Structural and Functional Profiling, and Spirulina Supplementing Effects on Pasta Matrix, Quality Evaluation of Gluten-free Pasta

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Supervised By: Dr. Muhammad Waqas Alam Chattha

A Thesis Submitted to the Atta-ur-Rahman School of Applied Bio-sciences in

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Science in Agribusiness Management

THESIS ACCEPTANCE CERTIFICATE

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ABSTRACT

DEDICATION

I dedicate this work to my parents, my beloved kids, and my dear husband Ali

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ABBREVIATIONS

SEM-Scanning Electron Microscopy

FAO - Food and Agriculture Organization

GHI - Global Hunger Index

GRAS - Generally Regarded as Safe

FDA - Food and Drug Administration

AOAC-Association of Official Analytical Chemists

CD-Celiac Disease

SP – Spirulina Powder

RF-Rice Flour

QF-Quinoa Flour

GAE – Gallic Acid Equivalent

TPC – Total Phenolic Content

AOA – Anti-Oxidant Activity

FC – Folin–Ciocâlteu

DGPF- Dehydrated Green Pea Flour

WPC-Whey Protein Concentrate

CPF- Chick Pea Flour

WHO -World Health Organization

ABSTRACT

This research aims to evaluate the outcome of total substitution of wheat flour with rice and quinoa flour which are considered as good alternate on starch, protein content, and physicochemical characteristics of gluten-free pasta. Gluten is a primary storing protein in wheat, it is predominantly a structural protein in wheat flour which gives stability, elasticity and firmness to the wheat derived products, such as bakery items, pasta, roti, noodles, and semolina.

It is gaining popularity among individuals who have gluten intolerance or celiac diseases. Thus far, a gluten-free diet has proven to be the most effective treatment for CD patients. Spirulina Arthrospira, a type of cyanobacteria that thrives in warm and alkaline waters, is highly sought after as an important element in functional food because of its abundant protein content, as well as a variety of vitamins, minerals, and antioxidants. Having a protein content of up to 70%, it is a great alternative for vegetarians and vegans. Spirulina's unique blue green color and antioxidant properties render it an excellent mineral supply, essential to the development of strong bones, teeth, and muscles. A comparative study is conducted to compare the supplementation effect of spirulina on gluten free pasta quality. Starch and proteins undergo progressive changes in their structure throughout the process of creating pasta. For this the structural and functional characteristic of the pasta matrix were investigated and the impact of supplementation was also determined. Purposes of the research was to obtain a more comprehensive understanding of how different components affect the quality of pasta and the underlying mechanisms involved. For these three different formulations were selected 5%,10%,15% of spirulina was added to gluten free flour of rice and quinoa, where quinoa was used because of its nutritional qualities and a binding agent. Its physiochemical testing was done to ensure the nutritional profile of the newly made pasta and scanning electron microscopy (SEM) analysis was done to see the structural changes and quality of the final product.

One-way ANOVA and the Tukey test were used for analyzing the data The results show that higher percentage treatments (10% and 15%) significantly impact protein, ash, fat, and phenolic content compared to the control sample. The 5% treatment has a significant effect on ash and fat content but not on protein, phenolic, or moisture content. The 15% treatment significantly affects moisture content, while the 5% and 10% treatments do not. The morphological characteristics of gluten-free pasta made from rice and quinoa, supplemented with varying percentages of spirulina, were examined using scanning electron microscopy (SEM). The control sample had a homogeneous structure with fewer voids and a denser matrix. The addition of 5% spirulina introduced significant changes, making the matrix more fibrous and less dense. The protein-starch matrix in gluten-free pasta is composed of various functional groups from proteins and starches. Higher spirulina concentrations lead to more disruptions in the protein-starch matrix, as the intricate structural network of gluten in wheat-based products is what essentially determines the quality of pasta. Furthermore, careful consideration is needed to optimize spirulina-fortified pasta formulations and manufacturing processes. Gluten intolerance is a crucial health problem that necessitates meticulous management and carries substantial implications for people, the healthcare system, and society at large. Enhanced consciousness and investigation in this domain can contribute to enhancing the quality of life for individuals impacted by this disorder.

CHAPTER 1: INTRODUCTION

Pasta, is a dietary staple in numerous nations, Pasta, once exclusive to Italian and European cuisine, has gained global popularity due to Italian immigrants in the early 20th century. It has been assimilated into local gastronomy in various countries, including Chinese, Japanese, Greek, Southern, and Brazilian cuisines. Pasta is categorized into long and medium-length types, flat, spiral, cylindrical and ribbon-cut pasta, quick extruded pasta, and small pasta used in soups. Ravioli, a type of pasta filled with various ingredients, is a popular choice in many dishes (Serventi & Sabban, 2002). Gluten-free pasta is gaining popularity among individuals who have gluten intolerance or celiac diseases Traditional wheat-based pasta relies on gluten, the primary structural protein in wheat flour, it provides the desired texture and integrity to the pasta. However the lack of gluten might result in poor cooking quality, texture, and structural integrity, developing gluten-free pasta is a substantial problem. Celiac disease is a serious global health issue and is evidently a prerequisite for the development of gluten in the diet.

Although initially it was reported from Caucasian countries but now it has been reported in other parts of the world. Among 10 most populous countries in the world there is only four countries have recorded the data on Gluten diseases, these are India, USA, Brazil, Russia. Other six countries Pakistan, Bangladesh Indonesia Japan, Nigeria is lacking the recoded data. As Pakistan have a same diet pattern and staple food based on gluten still it is under diagnosed.

To address this issue, alternative flours have been explored, such as rice and quinoa, in combination with various additives to increase the physicochemical properties of gluten-free pasta. Rice and quinoa flour are considered as healthy alternative ingredients in gluten-free pasta recipes due to their beneficial characteristics. One promising ingredient is Spirulina, a type of microalgae, which contains a high protein concentration of up to 70% is used as additive in fewer food products, nutritionists often regard it as an unconventional source of dietary protein (Adiba et al., 2011).

Algae could play a significant role in helping developing countries to achieve food self-sufficiency and it also has the potential to be used for treating certain ailments. Several microalgae species, including spirulina, chlorella, and Daniella salina, are grown and utilized as dietary supplements. It possesses a high abundance of proteins, vitamins, minerals, and antioxidants. Algae-derived supplements are widely favored for their possible health advantages, such as enhancing immunological function, promoting cardiovascular well-being, and offering anti-inflammatory characteristics.

Consumers' need for natural, sustainable, and nutritionally-rich food options is driving the growth and incorporation of algae and its derivatives in food items. Algae's adaptability renders them a vital component in the food sector. Spirulina, a microalga rich in nutrients, and Quinoa, a seed crop abundant in plant-based proteins and minerals, have both been studied as potential additives to enhance the nutritional composition of gluten-free pasta. The study will provide insights on optimizing gluten-free pasta formulations supplemented with spirulina and quinoa to improve the nutrient profile without compromising quality. This can help offer more nutritious options for people requiring gluten-free diets. Investigating the physicochemical properties, structural alterations brought about by processing, and starch-protein matrix of this novel gluten-free pasta formulation made with supplements of rice and quinoa flour was necessary for this objective to be achieved. Scanning electron microscopy (SEM) research was performed to study the impact of spirulina augmentation its structural integrity and shape, and the newly developed protein-starch matrix of the gluten-free pasta.

1.1 Importance of Gluten Intolerance and Demand for Gluten-Free Products

Wheat intolerance, or celiac disease, is a significant health problem which has attracted a lot of attention lately. Below are few crucial aspects about the significance of gluten intolerance Celiac disease is believed to impact approximately 1% of the worldwide population, although a significant number of cases may go untreated. With the rise in awareness the number of people getting tested and diagnosed with gluten intolerance has increased. Gluten consumption can induce an immunological reaction in persons with celiac disease, leading to harm to the small intestine. These symptoms can arise, encompassing abdominal pain, bloating, diarrhea, malnutrition, and an elevated susceptibility to various autoimmune conditions. The sole efficacious remedy for celiac disease is a rigorous diet devoid of gluten. It can be difficult, as gluten is a prevalent component in numerous dietary items, necessitating meticulous examination of labels and avoidance of specific products. Improperly managing a gluten-free diet can result in deficits of specific nutrients, including fiber, iron, and B vitamins. Prudent diet preparation and the addition of supplements may be required to preserve peak health.

Societal Impact: The rising occurrence of gluten sensitivity has resulted in a higher need for gluten-free products, prompting modifications in the food sector and greater awareness among consumers, healthcare professionals. Current research is investigating the fundamental origins of celiac disease, prospective alternative therapies, and the lasting health consequences of gluten intolerance. These technological breakthroughs have the potential to enhance the provision of healthcare and enhance the overall well-being of individuals impacted by them.

Gluten the main storage protein in wheat, is used to make a variety of products including flour, roti, noodles, and pasta. When people with celiac disease eat gluten-containing diets, they have a condition known as small intestine villi atrophy. Numerous symptoms result from this, including anemia, weakness, cramping, bloating, nausea, diarrhea, weight loss, and vitamin and mineral deficiencies (Menon et al., 2016). Products without gluten that are visually appealing, have a long shelf life, and are high in nutrients are more likely to be accepted and trusted by consumers. Patients with CD respond best to a diet that completely eliminates gluten, a protein present in wheat, barley, and rye. For CD patients, naturally gluten-free cereals such rice, maize, and sorghum are advised (Giuberti et al., 2015). Prior to its use as a traditional grain-based food item, dry pasta composed of durum wheat (Triticum durum) semolina was widely consumed due to its taste, convenience, and nutritional value. Additionally, due to its unique color, flavor, and cooking quality, durum wheat semolina was the perfect raw material for pasta manufacturing. The issue, however, was that there was a very small selection of healthful foods accessible for athletes, the obese, and those with celiac disease. Additionally, the gluten-free food products on the market provided less nutrition and were deficient in essential elements when compared to their wheat-based counterparts. Accordingly, there has been a rise in the need in recent years for the development of nutrient-dense gluten-free goods (Petitot et al., 2009).

Flours Supplementation: Gluten free flours used worldwide include Amaranth, buck wheat, white and brown rice, quinoa, maize, potato flour, oats millets, Tamari lentils which are being used to make gluten free products like cereals and bakery items. However, in Pakistan very few are available in the market leaving no other option to buy from Amazon or other online stores.

Maize, a gluten-free cereal crop, offers essential nutrients like carbs, protein, and vitamins. However, it lacks lysine and tryptophan amino acids and still needs supplementation. High-quality protein maize (QPM) is produced to improve its protein quality of maize, which is double the amount of tryptophane and lysine.(Maqbool et al.,2021).

Microalgal-based functional and nutraceutical products are gaining popularity worldwide because of their associated health claims. Several studies have proved that introducing Algae into cereals can enhance their protein content, as Algae are a rich source of vital amino acids and various types of Algae have been used as therapeutic agents for different health risks. Previous studies have verified that the nutritional and immune conditions of individuals can be improved by including most nutritious plant based super foods in the diet. Including microalgae in conventional diets can be a useful way to acquire functional nutrients. (Bashir et al., 2017**).**

Previously Youssef M. Riyad (2020) carried out a study on individuals with celiac disease. They fortified gluten-free noodles produced from potato flour by adding 5, 10, and 15% spirulina algae to increase its nutritional content. They examined the chemical composition of the spirulina algae, potato flour, and gluten-free noodles. They also assessed the in vitro protein digestibility and the amounts of vitamins, minerals, heavy metals, amino acids, and pigments. The sensory evaluation's findings suggested that spirulina algae powder be added to potato flour at up to 15% of the flour since the enriched noodles performed better than the control sample in every assessed sensory category. Due to lack of gluten Buck wheat is crumbly in nature, to increase its quality it can be used by combining it with other flours, but the chances of cross contamination increase during processing. Oat flour is also more prone to contamination because the absence of gluten, it makes the end product more moist.

Traditional wheat-based pasta relies on gluten to give the desired texture and integrity to the pasta. However, the no gluten pasta supplementation poses a great challenge to the food scientists, as the absence of gluten can lead to poor cooking quality, texture, and structural integrity. To address this issue, alternative flours have been explored, such as rice and quinoa, in combination with various additives to enhance the physicochemical profile of gluten-free pasta**.**

1.2 The Function and Significance of Ingredients

The ingredients chosen for the recipe play an essential role in creating the pasta and influencing the finished product's flavor, texture, and nutritional makeup. Most traditional pasta is manufactured with durum wheat, a kind of wheat. Protein and gluten abound in durum wheat, giving the dough the essential structure and flexibility. It creates a stable consistency that cooks without deteriorating. (Koli et al., 2022).

1.2.1 The Importance and Function of Rice

Rice flour is a conventionally used gluten-free flour used in pasta production because of its neutral flavor, ease of availability, and relatively low cost. Research has shown that rice flour-based pasta exhibits improved cooking quality, including higher water absorption, better firmness, and reduced stickiness compared to pasta made solely from other gluten-free flours, such as corn or potato (Foschia et al., 2017).

The average amount of rice consumed by each individual worldwide normally remains unchanged in recent years just a slight increase has been observed. The nutritional composition exhibits variation across different regions of the rice grain. The primary soluble carbohydrates are mostly starch which are predominantly present. The fiber component is resistant to digestion and is found in the bran and hull. Starches consist of amylose and amylo pectins. Protein is of superior quality; however, it lacks the essential amino acid lysine. When ingested in combination, the protein quality is enhanced as a result of mutual supplementing. The predominant lipid content (1-2%) of rice is mostly found in the bran portion. The primary fatty acids present are linoleic, oleic, and palmitic acid. It also contains α-tocopherol (vitamin E), enzymes, and pigment.

Proteins: Rice grain's protein content varies, with lower levels in embryo, scutellum, and aleurone layers compared to endosperm, pericarp, and testa. The protein composition includes albumins, globulins, protamines (gliadins), and glutelins. The protein composition varies among different cereals. Rice consists of 15% globulins, 5-8% prolamines, and the remaining portion is composed of gelatins. The gliadins and glutelins are collectively referred to as gluten proteins. Gluten exhibits distinctive elasticity and fluidity characteristics. Rice has a lower gluten concentration compared to wheat. Therefore, wheat is utilized in the production of dough, as well as in bread and other bakery items. Rice has a protein content of 7%. The endosperm protein is specifically located within protein bodies. Glutamin is present in high concentrations in crystalline protein bodies, whereas prolamine is present in high percentages in large spherical protein bodies. The rice grain's bran layer contains many of the B vitamins in high amounts. It also contains α-tocopherol, a vitamin E derivative.

Moisture: Rice grain moisture content ranges from 22%-38%, unfavorable for storage. Rice's enzymes, including amylases, proteases, lipases, oxidases, peroxidases, and phenolases, decline during storage. Cooked rice's sticky consistency is due to active alpha amylase enzymes, while its color comes from Anthocyanins and carotenoids.

Milling significantly alters rice's composition, reducing non-starch components and affecting its nutrient content. It removes the outer bran, containing essential nutrients, and primarily thiamine and lysine vitamins. This results in a low nutrient diet, making it insufficient for individuals. Milling also removes fat, fiber, minerals, ash, and vitamins, and is crucial for rice preservation. (Raajeswari., Food and Nutrition)

1.3 The Importance and Function of Quinoa

Quinoa flour, on the other hand, is a nutrient-dense alternative that provides a source of good quality protein, fiber, and essential nutrients. Studies have demonstrated that the inclusion of quinoa flour in a pasta can enhance the protein content and improve the textural properties, resulting in a firmer, less sticky, and more cohesive pasta structure (Bouasla et al., 2017). Quinoa, scientifically known as Chenopodium quinoa wild, is a type of annual herbaceous flowering plant. Quinoa has become widely famous globally primarily because of its appealing nutritional composition. Furthermore, the significant genetic variety of this resource holds immense potential for enhancing food security. It is known for its exceptional nutritional and functional features, mostly because to its high-quality protein content. This protein contains a diverse range of amino acids, with a notable abundance of lysine. Quinoa seeds are a rich source of protein, with chenopodin making up 37% and 2S albumin accounting for 35%. Quinoa have low proportion of prolamins (0.5-7% of total protein), making them suitable for celiac disease patients. Quinoa protein can be modified through enzymatic, chemical, and physical methods, making it a promising food source. (Dakhili et al., 2019).

Starch is essential for the functional characteristics of quinoa and its associated dietary items. Around 70% of the dry matter of quinoa grains is made up primarily of starch. The amylopectin in quinoa has both substantial quantities of short chains and extremely long chains. The distinctive characteristics of this starch have sparked research interest in its utilization for food and various other applications, including the production of Pickering emulsions**.** (Li & Zhu, 2018).

Eliana Pereira (2019) evaluates the nutritional value of Chenopodium quinoa Willd species quinoa grains of diverse color variants (black, red, and white) from different origins, by analyzing the nutritional value and chemical composition of three quinoa varieties, revealing proteins and carbohydrates as primary nutrients, the key hydrophilic compounds are sucrose and oxalic acid,.The three quinoa varieties had nearly identical characteristics.

Quinoa lack gluten, it is considered as functional food with high fatty acid content, vitamins, minerals, dietary fibers, and high amino acid content. Studies have shown that these seeds contain a diverse range of bioactive compounds like carotenoids, vitamin C, and phenolics, which can protect against diseases like cancer, allergies, and inflammatory ones, they may reduce the risk of cardiovascular diseases. (Gómez-Caravaca et al., 2014, Nowak et al., 2016). (2014, Abderrahim et al. in 2015, Tang et al. in 2015, Aziz et al. in 2018, and Pellegrini et al. in 2018.

A study validates the suitability of consuming various varieties of Chenopodium quinoa, including protein, fat, ash, carbohydrates, energy, organic acids, free sugars, fatty acids, and tocopherols. Quinoa can be consumed by fermenting it into a beer, sprouted for salads, and used as a nutritious feed for cattle. Quinoa seeds consist of resistant starch content can also be used as flour by increasing the texture of dough when mixed with the other flours as it is more denser and compact than the wheat and many bakery items and cereals can be made out of it. (Gómez-Caravaca et al., 2014) (Vilcacundo and Hernández-Ledesma, 2017).

1.4 The Importance and Function of Spirulina

Artherospira maxima and Arthrospira platensis are two species of photosynthetic bacteria that have been used as food sources for millennia. Spirulina, a dried biomass of A. platensis, is a multicellular and filamentous blue-green microalga (cyanobacteria) with antioxidant properties linked to a potential reduction in cardiovascular diseases and cancers. It is widely used as a dietary supplement and has potential advantages in gluten-free pasta production. Spirulina is recognized for its nutritional value and health benefits due to its abundance of macro- and micro-nutrients. It is used in various food products, including sports supplements, bakery items, drinks, dairy products, snack sources, and confectioneries. Algae like Spirulina and Chlorella are also used as natural colorants in food products, providing vibrant green and blue-green hues. (Jensen et al., 2001)

Incorporating spirulina into pasta could enhance the nutritional security and well-being of underdeveloped rural areas, address hidden hunger, and improve the nutrition of infants and children. Spirulina is considered safe for ingestion due to its beneficial constituents, including phycocyanin, β-carotene, xanthophyll pigments, α-tocopherol, and phenolic compounds. It consists of approximately 50-70% protein, 20% carbohydrates (polysaccharides), 5% lipids, and 7% minerals. Additionally, it contains substantial amounts of phenolic compounds, which possess antioxidant properties that have been linked to a potential reduction in the risk of cardiovascular diseases (CVD) and cancers, as indicated in previous studies (Hernández-López et al., 2023).

The World Health Organization (WHO) has classified them as superfoods because of their abundance of bioactive chemicals that have potential biological effects. Due to the growing recognition of the health benefits and lifestyle problems, microalgae are being given fresh focus in the fields of nutrition and food science.Spirulina has the potential to enhance the nutritional composition and technological features of bread due to its high protein content and biological activity. However, adding additional constituents to a dough matrix can be challenging. Supplementing pasta with more than 7.5% Spirulina biomass can improve its nutritional value and antioxidant activity, particularly in terms of phenolics and flavonoids. Furthermore, findings from several studies indicate its application in food formulations, particularly in sports supplements, bakery items, drinks, dairy products, snack sources, and confectioneries. Some algae, such as Spirulina and Chlorella, are used as natural colorants in food products, providing vibrant green and blue-green hues. These algae-based colors are increasingly used as alternatives to synthetic food dyes, which are facing growing consumer concerns. Certain types of algae, such as agar, carrageenan, and alginates, are widely used as thickening, stabilizing, and gelling agents in a variety of food products like jellies, james,ice creams.(Ahmad et al.,2023).

NASA uses spirulina for astronauts and natural food additives, including pasta. Its rich nutritional composition and disease-fighting properties make it suitable for various food formulations. Spirulina could enhance nutritional security in underdeveloped rural areas and address hidden hunger in infants and children. Microalgae, rich in omega-3 fatty acids, are often used in food products, including supplements and infant formula. Further research is needed to explore its potential in the food additive sector. (Koli et al., 2022)

1.4.1 Sustainability Advantages

Spirulina has been suggested as a possible substitute ingredient due to its remarkable nutritional composition, minimum environmental impact, and capacity to decrease food waste. Research conducted by (Habib et al., 2008) has shown that When it comes to protein sources derived from animals, spirulina has a lower carbon footprint, making it a more environmentally friendly choice. Additionally, it is possible to utilize waste streams, such as waste water, for the cultivation of Spirulina. This practice has the potential to reduce food waste and boost resource efficiency (Habib et al., 2008).

This study explores the potential of incorporating Spirulina into pasta, a popular snack, to enhance its nutritional value and health benefits. It will also evaluate the physical and chemical properties of the enhanced pasta and its long-term viability. Additionally, it will assess the effectiveness and long-term viability of utilizing Spirulina as a substitute ingredient.

1.4.2 Toxicity and Safety

Spirulina is a type of cyanobacterium, some of which can create toxins such microcystin. Certain spirulina supplements have been discovered to contain microcystin, while the quantities are low. Microcystin can induce gastrointestinal symptoms, including diarrhea, flatulence, headache, myalgia, face flushing. Chronic exposure to even small amounts of microcystin can have harmful effects on multiple organ systems due to the potential for heavy-metal toxicity .(Bhattacharia, 2020) additionally observed spirulina's ability to safeguard humans from arsenic toxicity. The Chinese State Food and Drug Administration (CSFDA) has identified lead, mercury, and arsenic contamination in commercially available spirulina supplements, despite the fact that doses ranging from 10 to 19 grams per day have been proven safe over many months.

This study examines the physical and chemical properties of gluten-free pasta made from rice and quinoa flour with varying Spirulina percentages. The study evaluates the functionality and sustainability of using Spirulina as an alternative ingredient. SEM Scanning Electron Micrography analysis is used to compare the surface topography, crystallographic orientation the structure of the un-cooked pasta revealing the impact of processing on the product by Lucisano et al. (2008) and Zweifel (2001).

1.6 Objectives

1. The purpose of the study is to evaluate the physiochemical properties of gluten free pasta

2. The study aims to analyze the impact of ingredients on pasta quality, standardize formulations, and understand the structural and functional profiling of the pasta matrix.

CHAPTER 2: LITERATURE REVIEW

There has been growing demand for better dietary choices, and researchers to investigate alternate ingredients for making pasta dough, research by Gałkowska et al. (2021) has shown that incorporating alternative grains like quinoa and spelt into pasta dough can enhance its nutritional profile, providing a healthier alternative to standard wheat-based pasta options, thereby meeting growing demand for better dietary choices. (Kaur p.,2023).

2.1 Fortification in Gluten Free Products

The growing awareness of gluten-related illnesses presents a promising potential in the global market for gluten-free products. A greater number of businesses are making efforts to get into this niche industry, the demand for gluten free products rises in the conjunction with the number of persons with CD increases. The GF market is growing at the rate of 8.7% per year. There was 1.7 billion $ market in 2020 and expected to reach 9.2 $in 2030 (Gluten-free products market size and share report, 2030).

The study investigates the use of sweet potato flour in gluten-free spaghetti, aiming to increase protein content by adding whey protein concentrate and chickpea flour. The study aimed to increase the protein content of the spaghetti by adding whey protein concentrate (WPC) and chickpea flour (CPF) at rates of 5%, 10%, and 15% respectively, compared to a control sample. The results show that adding WPC and CPF increases optimal cooking time, weight, and volume without impacting cooking loss. The spaghetti with 10% WPC and 10% CPF had the lowest starch digestibility, lowering glycemic index. The addition of these ingredients enhances spaghetti's protein content, attractiveness, and functional properties. Furthermore, the addition of whey protein concentrate (WPC) and casein phosphopeptides (CPF) to spaghetti can greatly enhance its protein content, as well as improve its overall attractiveness and functional properties (Giri & Sakhale, 2021).

Instant noodles, a popular convenience food, have been studied for their nutritional value and health effects. A study found that 2-10 % mushroom powder supplementation increased cooking time, water absorption, and tensile strength, while protein content increased significantly. The study also found that when 6% of mushroom powder was added to the noodles it had enhanced protein content to 11.3% and fiber content to 1.96%, with optimum cooking quality and sensory characteristics. Comparing the optimal product to control, it had 17.3 and 8.89% higher protein and fiber.

This innovative technology can cater to double income families and offer a nutritionally superior, convenient, and high-acceptability instant cooking product (Arora et al., 2017).

Limroongreungrat and Huang (2007) used alkaline treated sweet potato flour as an alternate to wheat flour for pasta production. Pearl millet cereal is used to make gluten free flour for pasta, it has a unique flavour, a rich source of minerals, protein, carbohydrates and fats. Depigmentation method was also used for pearl millet pasta production, finding improved protein and starch digestibility of nutritious pasta. Pearl millet contains oxalates which interfere with food absorption leading to kidney stones, it is also not recommended thyroid gland disorders. Rathi et al. (2004)

Osorio-Diaz, Zanita Ugaric-Hardi, and Marko Juke, conducted a study in 2008 to examine the quality parameters of noodles that were prepared using different supplements. Split out maize flour, defatted soy flour, mixtures of maize and soy flour, lecithin, and wheat straw were among these supplements. The noodles prepared using split out maize flour, maize flour, and wheat straw additions obtained the highest overall sensory rating. These samples had cooking losses of less than ten percent. In a study conducted by Maud Petitot et al. (2010), the researchers examined how adding bean flour to pasta affects its composition and the resulting impact on the digestibility of starch when tested in vitro. The incorporation of a significant proportion 35% of legume flour, namely split pea flour, resulted in slight modifications to the structure of the pasta. In(2010, M. L. Sudha and K. Leelavathi) conducted a study that investigated the effects of additives like dry gluten, sodium stearoyl lactylate (STL), and glycerol mono stearate (GMS) on the rheological characteristics and pasta-making quality of Indian Triticum aestivum. Amaranth grain is a pseudo cereal gluten free grain ,its very nutritious for individuals with celiac diseases, gastrointestinal cancers, anemia ,in a study conducted by (Bashir et al., 2012) 5–10% amaranth seed flour was incorporated in to cheese bread to the recipe to boost the product's protein and fiber content.(Andrea Dos et al.,2012)

The study investigated the effects of varying concentrations of whey protein concentrate (WPC) 5%, 7.5%, 10% and additives on the quality of vermicelli made from Indian durum wheat. Results showed that increasing WPC increased the weight of cooked vermicelli and increased cooking loss. The sensory evaluation revealed that adding more than 5% WPC resulted in a pale color, soft texture, and a sticky mouthfeel. Adding a combination of additives reduced cooking loss, created a creamy yellow color, and improved the vermicelli's texture. The vermicelli with 5% WPC and a combination of additives had a protein content of 16%, while the control vermicelli had a protein value of 11.5%. A scanning electron microscopy analysis was conducted on three types of vermicelli: control vermicelli, vermicelli with 5% whey protein concentrate (WPC) revealed that the vermicelli with 5% WPC had a rough surface with noticeable rupture, while the vermicelli with additives had a smooth structure. (Prabhasankar et al., 2007).

The study evaluated the impact of different herbs on pasta made from semolina. Pasta was fortified with herbs at different concentrations, 2.5%, 5%, 7.5%, and 10%. showing significant differences in functional characteristics, cooking quality, bioactive profile, antioxidant potential, nutrient digestibility, and color. The findings suggest herbs have potential as food ingredients in value-added cereal products. Changes in the molecular and structural composition of the pasta with 5% herb blend and the control pasta were shown by Fourier Transform Infrared and Scanning Electron Microscopy. Agglomerative hierarchical clustering and principal component analysis were also employed to confirm the variation in the integration of data and herbs. (Bhandari et al., 2022).

2.2 The Structure and Composition of Rice

Starch, a complex combination of amylose and amylopectin, is stored in plants as granules which have different shapes like angular, oval, round, spherical, or irregular (Linde boom et al., 2004) Rice starch granules are the smallest among cereal grains, measuring 3-8μm in diameter. Starch granules contain lipids, proteins, minerals, and moisture, with non-waxy rice having 0.3-0.4 % lipids and on the other hand waxy rice having 0.03%. **(**Morrison and Azurin, 1987, Morrison et al., 1984).

2.2.1 Functional characteristics of rice starch

The adhesive properties and usefulness of rice are mostly governed by the starch component. When uncooked rice is cooked, it goes through a number of changes, such as glass transition, swelling, gelatinization, pasting, amylose leaching, and degradation of the starch granules. The desirable textural characteristics of the cooked grain are the result of these procedures. Gelatinization is the process in which native starch undergoes collapse when heated with an adequate amount of water. (Fitzgerald, 2004).

The bran component of rice has the majority of its fat content, which ranges from 1% to 2%. The lipid particles are present in the aleurone layer and account for 20% of the dry weight. There is a large amount of lipids in the protein bodies, particularly in the central region. Linoleic, oleic, and palmitic acid are the three main fatty acids.

The rice contains a range of 29-42% as linolenic acid, which is an important type of fatty acid. The EFA (Essential Fatty Acid) levels rise in correlation with increasing temperatures during grain development, while simultaneously causing a decrease in oil content. The Starch lipids mostly consist of monoacyl lipids that form a compound with amylase. The lipid content in waxy starch granules is minimal. Starch lipids make a minimal contribution to the energy content of rice grains (Rajeswari, Food and Nutrition).

2.3 The Structure and Composition of Quinoa

Quinoa, also known as Chenopodium quinoa Wild., is categorized as a pseudo grain and is recognized as a complete food due to its 15% high-quality protein content and ideal amino acid balance. Quinoa is a type of grain that is native to the Andean region. Since it is not a true cereal grain, it is categorized as a pseudo cereal. At its fresh weight, quinoa has a protein content of 14.6%. This protein is exceptionally well-made, with 3.2% and 6.1% of its protein composition coming from high concentrations of histidine and lysine, respectively. Fresh debittered quinoa has PER levels between 78 and 93% of casein, whereas cooked quinoa can have PER values between 102 and 105% of casein. With 44–77% of the total protein content in quinoa coming from albumins and globulins, they are the main source of protein. Quinoa may be gluten-free due to the trace amounts of prolamins (0.5–7.0%) that are present. Quinoa contains a large number of antioxidants, particularly polyphenols. When it comes to bioactive compounds, it has the highest concentration of any pseudo-cereal crop. Additionally, studies have indicated that quinoa is considered gluten-free, which means that people with wheat allergies and celiac disease can use it. (Sohamy et al., 2018).

Around 5.6% of the fresh weight of quinoa is made up of fat, of which 55–66% is composed of the essential fatty acids linoleic and r-linolenic acids. Because quinoa oil has relatively high quantities of natural antioxidants in its raw form (690-754 ppm of a-tocopherol and 760-930 ppm of y-tocopherol), it is extremely stable. In refined oil, these values drop to 450 ppm and 230 ppm, respectively. Given its high oil quality and the existence of varieties with up to 9.5% fat content, quinoa may prove to be a lucrative new oil crop.

Although grains contain between 1 to 12% of their weight in starch, this starch contains very little amylose. The majority of the quinoa's starch granules range in size from 0.7 to 3.2 pm. But unlike other small granule starches, such as rice, quinoa begins the gelatinization process at a much lower temperature—roughly 58°C. The "earthy" flavor of quinoa may be partially attributed to its high levels of polyamines, which range from 22 to 18-2690 nmol/g fresh weight, but they are not likely to be the primary cause. In comparison to barley, rice, or wheat, quinoa has greater concentrations of riboflavin (0.39 mg), a-tocopherol (5.37 mg), and carotenes (0.39 mg) per 100 g of dry weight. But its niacin content is barely one-fifth (1.06 mg) of that of the original. Quinoa contains 0.20 mg (10% of the recommended daily allowance) of vitamin B6, 0.61 mg (9–15% of the RDA) of pantothenic acid, 23.5 pg (12% of the RDA) of folic acid, and 7.1 pg (7–24%) of biotin. Amounts of other vitamins that are less than 10% of the RDA are present. When compared to other grains, quinoa has higher contents of calcium (1487 mg), iron (132 mg), potassium (9267 mg), magnesium (2496 mg), copper (51 mg), manganese (100 mg), and chloride (1533 mg) per 1 kilogram of dry weight. Sodium to potassium is a ratio of 1:76. A 100-gram serving of quinoa supplies 27–40% of the Recommended Dietary Allowances (RDAs) for iron (Fe), 23–76% for magnesium (Mg), 47–2000% for copper (Cu), 1–16% for phosphorus (P), 15–19% for potassium (K), 10-15% for zinc (Zn), and only 1-2% for sodium (Na), depending on age and sex. The main antinutritional substances in quinoa are saponins. Dehulling, abrasive technique, or washing are two ways to get rid of these compounds. A 100-gram portion of quinoa contains little more than 0.01 grams of saponins. When compared to spinach leaves, quinoa leaves have higher levels of fat (1.8%), ash (3.3%), fiber (1.9%), nitrates (0.4%), vitamin A (2085pg RE/100 g), vitamin E (2.9 mg (u-TE/100 g), Mg (83 mg/100 g), and Na (289 mg/100 g). It is a notable source of vitamins and minerals, and it has also been shown to contain compounds including flavonoids, phytosterols, and polyphenols that may have nutraceutical benefits. The material has a number of technological properties that allow for a wide range of applications, including solubility, water-holding capacity (WHC), gelation, emulsifying, and foaming. Additionally, because of its high vitamin E and omega-6 fatty acid concentration, it has been labeled as an oil crop. The physical and chemical attributes of quinoa starch include stickiness and freeze resilience. (Koziol, 1992).

Quantification of Flavonoids and Phenolic Compounds: Phenolic compounds, among other phytochemicals, are present in all plant-based diets and affect the nutritional value and flavor of these food items. The results of this experiment showed that the total phenolic content of the quinoa flour was 17.86±0.49 µg GAE/g dry weight. Given their potential to improve human health, polyphenols are essential for human diet. Their functions as antioxidants, anti-inflammatory agents, antimicrobials, and cardioprotective agents are primarily responsible for this. Additionally, polyphenols play a critical role in preventing diabetes mellitus and neurological diseases. Quinoa has also been found to have antioxidant, antiviral, allergenic, anti-platelet, and anti-tumor effects. (Sohamy et al., 2018).

The water and oil absorption capacity of food components are crucial functional qualities that enhance mouthfeel and preserve flavor. The protein level is important for water absorption in foods like soups, dough, and baked goods are examples of viscous foods where adding quinoa flour to these recipes could be beneficial. Quinoa flour's had (46%) oil absorption capacity which was lower than that of African yam beans (110.25–132.82%), soy flour (84.4%), and wheat flour (84.2%). Because it preserves flavor and improves the texture of food in the mouth, oil absorption is essential.

(Kinsella, 1985).

2.4 The Structure and Composition of Spirulina

Spirulina, a nutrient-dense blue-green algae has been investigated for its potential to enhance the nutritional profile and functional properties of gluten-free pasta.

Prior to 1962, spirulina was classified as a form of algae. Nevertheless, in that particular year, it underwent a reclassification and was accepted as a constituent of the prokaryotic kingdom. Following that, the term "blue-green algae" was designated. The genus Arthrospira have the ability to create filaments or spiral trichomes of varying sizes. Spirulina exhibits a spectrum of flexibility, allowing it to assume various shapes, from tightly coiled to straight and unwound. The filaments usually have lengths ranging from 2 to 12 µm, although occasionally they can reach up to 16 µm. The thread's diameter varies between 3 and 12 µm, and the filament's cells contain gas vacuoles that assist in flotation (Nawal at al.,2022).

Spirulina contains all the necessary amino acids especially those that our body requires. S. Arthrospira, which is very rich in proteins, comprises of 53 to 68 percent of the cell's dry weight. Its protein has all the vital amino acids. Arthrospira also possess substantial amounts of polyunsaturated fatty acids (PUFAs), approximately 1.5–2 percent, and a total lipid content of 5–6 percent. These polyunsaturated fatty acids (PUFAs) consist of γ-linolenic acid (GLA), which is an omega-6 fatty acid. Arthrospira also contains vitamins, minerals, and photosynthetic pigments. Taken within a strict vegetarian diet, it provides lysine and methionine. Spirulina usually consists of a protein which is comprised of 55% to 70%, polysaccharides making up 15% to 25%, total fat accounting for 5% to 6%, nucleic acids comprising 6% to 13%, and minerals making up 2.2% to 4.8% (Hosseini et al., 2013). The branching polysaccharide found in A. platensis, which makes up 25% of its composition, exhibits a structural resemblance to glycogen. The algae have 1.5-2.0% of polyunsaturated fatty acids (PUFAs) in its total lipid content. The Arthrospira sp. is rich in γ-linoleic acid (GLA), comprising 30-35% of total polyunsaturated fatty acids (PUFAs). Additionally, it contains eicosapentaenoic acid (EPA), arachidonic acid (AA), stearidonic acid (SDA), and docosahexaenoic acid (DHA). GLA is a crucial fatty acid that is seldom found in components or food. In addition, Arthrospira sp. has a low cholesterol content of 32.5 mg per 100 g. For example, 10 grams of spirulina powder contains only 1.3 milligrams of cholesterol, but the same amount of protein from an egg would include 300 milligrams of cholesterol. Furthermore, the Arthrospira spp. is easily broken down since its cell walls lack cellulose so after 18 hours of ingestion over 85% of its protein is easily digested and absorbed (Sasson et al,.1997). The food component's low cholesterol and high nutritious content are essential to its performance. Arthrospira sp. contains a wide variety of minerals, including calcium, phosphorus, selenium, sodium, manganese, copper, iron, and manganese, as well as vitamins (B1, B2, B3, B6, B9, and B12) photosynthetic pigments, and proteins that resemble insulin. Consequently, the biomass derived from this abundant source of elements is utilized as additives in the agriculture, culinary, and pharmaceutical industries.

Despite its nutritional function, research has shown that certain chemicals found in Spirulina powder, such as c-phycocyanin, zeaxanthin, GLA, sulfated polysaccharides, sulfolipids, and insulin-like protein, have pharmacological activity and can have a clinically curative effect (Li et al., 2006; Soheili and Khosravi-Darani, 2011; Ou et al., 2012; Chen et al., 2014; Yu et al., 2012; Tanticharoen et al., 1994). The most intriguing biochemical molecule found in Arthrospira is c-phycocyanin, which exhibits anticancer properties by specifically inhibiting COX-2. This effect is attributed to its unique conformation and big size, as depicted in Figure 42.2. The arrangement of the c-phycocyanin enables it to effectively attach to the active region of COX-2 (Reddy et al., 2000) (Anusree et al., 2023).

Lutein is a carotenoid that is soluble in lipids and is acquired by humans through their diet. Zeaxanthin is synthesized by certain microalgae species such as D. salina, Spirulina, and Chlorella sp. It has a variety of recognized medicinal advantages, including helping to prevent macular degeneration, lowering the risk of heart attacks and strokes, and lessening the effects of various other serious metabolic diseases. Zeaxanthin and Lutein accumulate in the eye’s macula and serve as a blue light filter and antioxidant, which helps protect the eyes. Studies have shown that consuming lutein through the diet can prevent the occurrence of several conditions such as atherosclerosis. In addition, numerous research have explored the therapeutic benefits of lutein, demonstrating a reduction in the likelihood of age-related macular degeneration.

In another study it was observed that with the addition of 6% spirulina to the pasta there was a pleasant taste and flavor, and also remained structurally intact during cooking, showing no signs of splitting, disintegrating, or cracking. Furthermore, the product exhibited a firm texture, non-sticky surface, and minimal cooking loss. A study carried out in 2014 by Joshi et al. found that the end product's market approval was improved when spirulina powder and maize flour were combined to produce extruded crisps. In a separate analysis conducted by Bashir et al. in 2017, the rice soy crisp enriched with 6% spirulina (RSC-S6) obtained the highest acceptance rating of 8.50 from the judges (U.K. Veena et al., 2022).

2.4.1 Spirulina as a Potential Ingredient

Elderly people's nutritional state can be greatly impacted by old age, which can result in decreased appetites and increased requirements for nutrition. Nutritional deficiencies are common, leading to malnutrition, frailty, and morbidities. High protein deficiency among the elderly is a concern, as it contributes to health benefits such as faster rehabilitation for hip fractures, increased lean body mass, and reduced frailty risk. Aging is also connected to inadequate consumption of micronutrients like minerals and vitamins. The microalga Spirulina is known for its high protein content, vitamins, minerals, and antioxidant activity. However, specific products for the elderly with Spirulina biomass have not been developed. Studies show that Spirulina biomass can have medicinal properties, including anti-cancer, hypolipidemic, and protective actions against diabetes and obesity. Sensitivity and shelf-life determination are crucial factors in food development, as well as sensory acceptance and packaging properties. In order to meet the nutritional demands of the senior population, this study attempted to make chocolate flavored, shake-like powdered food that was enhanced with spirulina. (Santos et al,.2016).

2.5 Starch Protein Matrix

Gluten-free (GF) pasta’s demand has surged significantly because of the growing awareness of the detrimental health consequences of gluten-rich diets, particularly for persons with gluten sensitivity or celiac disease. The starch and newly added polymeric proteins in gluten-free pasta are the main components that hold much significance in determining the pasta's structural integrity, texture, and cooking qualities. Nevertheless, the drying temperature is a significant challenge in the manufacturing of gluten-free pasta, and is essential to fine-tune the conditions for drying to get the desirable attributes of gluten-free pasta. Effective management of heat and mass transfer processes is crucial in the industrial manufacturing of gluten-free pasta to guarantee enhanced quality and the intended physical attributes of the finished product. Therefore, it is essential to have a thorough understanding of engineering principles and use them effectively to achieve maximum production while minimizing any decrease in quality (Ahmed et al., 2024).

Wheat flour noodles which are a commonly consumed as a staple food in Asia, its starch protein matrix can be enhanced by substituting conventional wheat flour with HAW flour. This substitution has the potential to increase fiber consumption in a wide range of individuals. When comparing it to the wild type, HAW with an amylose content of up to 93% exhibited clear microstructural and molecular characteristics of starch, as previously described by Li, Dhital, Slade, et al. in 2019. These differences have the potential to cause structural alterations in the protein-starch matrix of HAW foods The results show that when the protein content was increased in native HAW flour it also enhanced the food integrity and thermal stability of starch in HAW noodles and hence, enhanced the ability to withstand α-amylase digestion.

The native grains exhibit many levels of starch-protein interactions, which arise from proteins present on the surfaces of granules and within internal pores. There are distinct protein bodies found surrounding starch in the grain tissue (Dhital et al., 2019). The interplay between carbohydrate and protein in food ingredients, such as wheat flour, and their reaction to processing, have significant ramifications for both the quality of food and its nutritional benefits (Li et al., 2021).

Starch, a significant digestible carbohydrate in human diets, is produced in a compacted semi-crystalline granular structure through the organized arrangement of two water-loving glucose polymers (amylopectin and amylose) during photosynthesis. The structure is hierarchical and may be divided into four levels of organization: lamellae, granular, growth ring, and molecular levels. These levels span a range of lengths from nanometers to micrometers (Zhang et al., 2015).

2.6 Scanning electron microscopy (SEM)

SEM, or scanning electron microscopy, is a versatile technique that is commonly employed for the analysis of specimen surfaces. The scanning electron microscope (SEM) may provide a detailed topographic representation of the material in a semi three-dimensional manner, thanks to the high depth of focus offered by its detector. The spatial resolution is influenced by various elements, such as beam spreading effects, the diameter of the incoming electron beam, signal to noise ratio, and instabilities. Increasing the voltage in the microscope enhances contrast and resolution, allowing for the use of thicker materials. Nevertheless, an increase in voltage can also result in the destruction of the sample. Ultimately, the resolution is contingent upon the caliber of the sample preparation as well. Typically, the specimen must possess conductivity or require the application of a conductive coating. A common method to achieve this is by depositing a gold coating onto the specimen by a process called sputtering, which simultaneously enhances the contrast. Alternatively, one can utilize low voltage scanning electron microscopy (SEM) as a means to prevent the accumulation of electric charge on the material surface (Heneen and Brismar, 2003; Langton and Hermansson, 1989) (Petitot et al., 2009). Moreover, it is necessary to exclude water prior to conducting SEM examination. The sample can undergo dehydration after a fixation stage or be solidified through freezing. Another constraint is the requirement for a specific level of vacuum purity to prevent the electron beam from interacting with molecules in the gaseous phase. The resolution threshold of a scanning electron microscope (SEM) typically ranges from 5 to 10 nanometers, while the scanned area usually spans from 50 to several hundred millimeters in each dimension. Scanning electron microscopy is extensively employed to comprehend phenomena in pasta. The initial papers employing SEM to examine the composition of pasta were published in the 1970s and 1980s (Dexter et al., 1978; Matsuo et al., 1978; Pagani et al., 1986; Resmini and Pagani, 1983). It is commonly employed to compare the effect of new ingredients on the structure, especially on the protein network. This has been studied by Aalami and Leelavathi (2008), Aravind et al. (2012a, d), Fuad and Prabhasankar (2012), Majzoobi et al. (2011b), Manno et al. (2009), Prabhasankar et al. (2010), and Sung and Stone (2005). Cunningham et al. (2007) employed structural equation modeling (SEM) to illustrate the impact of starch swelling on the surface of pasta during the cooking process.

In another investigation conducted by M. Alireza Sadeghi and S. Bhagya (2008), it was discovered that Mustard protein isolate (MPI) was utilized in pasta products as a supplement. The MPI was prepared by subjecting it to steam injection heating to eliminate antinutritional factors. Various levels of MPI, ranging from 0% to 10%, were incorporated into the pasta products. The study assessed the impact of different levels of supplementation on the rheological properties of pasta dough, as well as the chemical structure, cooking characteristics, nutritional value, and color traits of dried samples. The findings indicate that the scanning electron microscopy (SEM) analysis of various samples demonstrates that the addition of modified potato protein isolate (MPI) enhances the structure surrounding starch granules in pasta.

CHAPTER 3: METHODOLOGY

The research was structured into three distinct phases: sample preparation, physiochemical testing, and SEM and statistical analysis. Initially, the Gluten Free Pasta was prepared manually. A comparative analysis of the nutritional, chemical, and physical properties of pasta supplemented with spirulina was subsequently carried out.

This entailed subjecting both the control pasta and the pasta formulations supplemented with Spirulina powder to simultaneous physicochemical tests. The results obtained were analyzed to investigate the potential efficacy of Spirulina derived chemicals in an uncooked gluten-free pasta product. The testing was divided into two main categories, each assessing a distinct aspect of the food product: physical and chemical/nutritional testing. Then each of the formulation was subjected to SEM analysis to see the stability and integrity of the uncooked pasta. The test samples were tested in triplicates and were also subjected to duplicate testing, following the guidelines set by AOAC (Association of Official Agricultural Chemists) International.

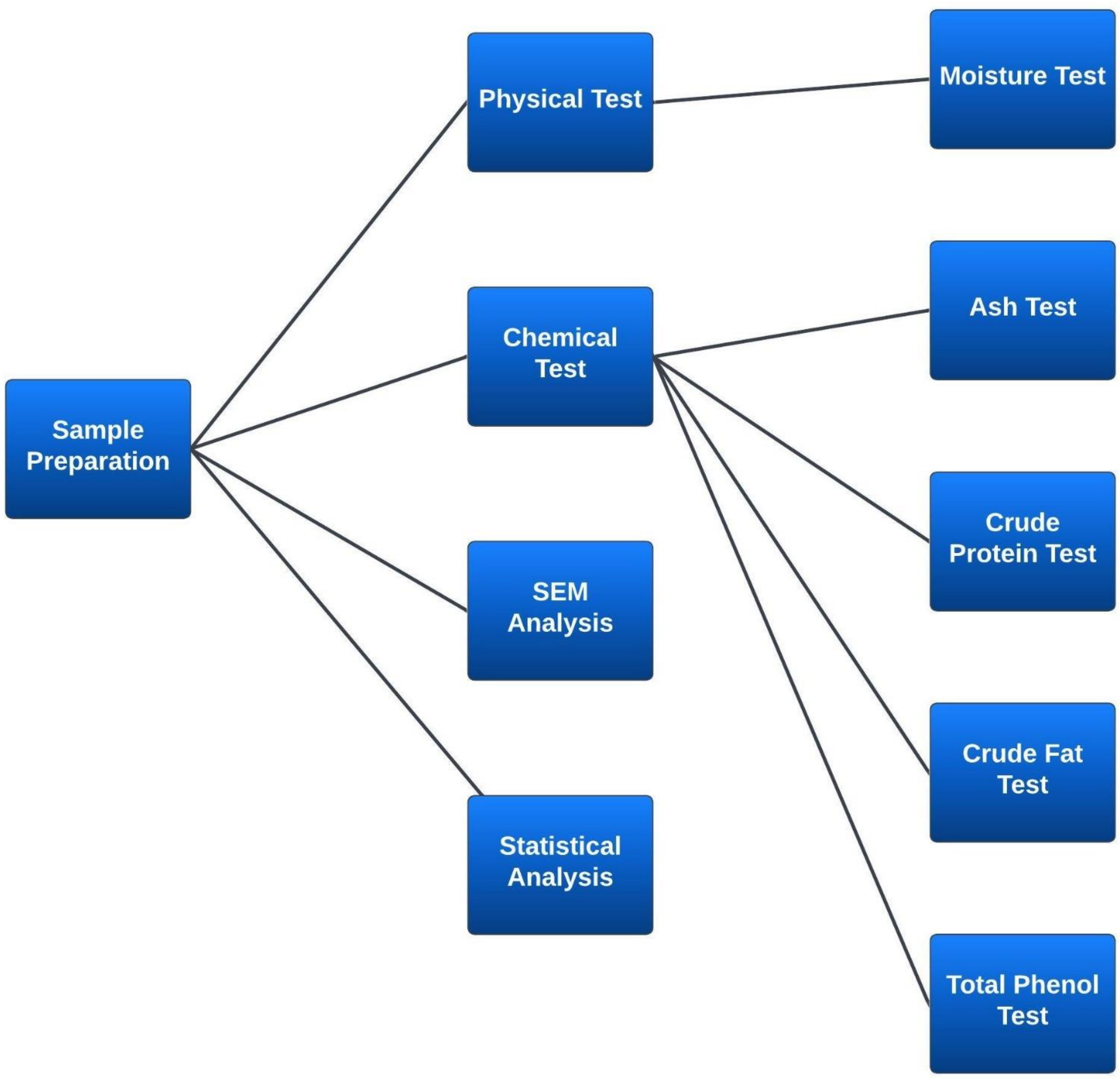

Fig 1: Research Layout

Above shown in Figure 1 is the experimental process flow displaying the methodology and tests performed. The source of the organic spirulina algae powder (SAP) was KN KATE NATURALS in California. Prior to further pasta processing, the powder was kept cool, dry, and out of the sun in a polypropylene bag coated with aluminum. The whole grain flour for organic quinoa was purchased from Bob's Red Mill Natural Foods USA. The rice flour came from Pakistan's Fauji Foods Limited. Every ingredient required to prepare the pasta was bought from Pakistan's local bazaar. When choosing these concentrations, the finished product's market economics were taken into account.

1. Blends Preparation

Firstly, Pasta dough was manually made using a recipe for 100 grams of flour, keeping rice flour, quinoa flour, and 60 ml of water constant in all formulations and incorporated 5%, 10%, and 15% levels of SAP spirulina algae and created a dough of homogenous mixture.Fig.2. The sheets were made out of dough balls and imperiled to Marcato Ompia 150 pasta making machine. Each formulation was subjected in triplicates for physiochemical testing and SEM analysis.
Samples were stored in an airtight container and kept at room temperature until used.

Fig 2: Spirulina Samples

Table 1 : Blend Preparation:

SPIRULINA POWEDER (SP)	control	5%	10%	15%
RICE FLOUR (RF)	70%	70%	70%	70%
QUINOA FLOUR(QF)	30%	25%	20%	15%
WATER	30ml	30ml	30ml	30ml

3.1 Physical Tests

3.1.1 Moisture Content

The moisture content of food items is determined because it may affect the period of time the food remains consumable. By analyzing the moisture levels in a product, the risk of deterioration can be quickly assessed and the growth of microorganisms in the food product (Zambrano et al., 2019). Furthermore, the moisture level possibly impacts the processing of food goods too. An excessive amount of moisture might result in the product becoming adhesive, whilst insufficient moisture can cause the product to become rigid and fragile. Moisture content tests are employed in commercial settings to assist producers in optimizing their processing parameters in order to attain the required texture and consistency of the product (Neilsen, 2017). Moisture content of pasta should be 12% as determined by

The weight of the petri dish and lid was determined when they were empty. Approximately 2-3 grams of the sample was measured and placed it in the dish i.e., the initial weight W1. The sample was spread evenly using a spatula. The dish containing the sample was placed inside the oven until the sample reached a constant weight. To ensure accurate results, a constant temperature was carefully maintained by avoiding the opening of the oven door while the samples were drying. Allowing it to dry for three hours at a temperature of 105 degrees Celsius. Once the dish was dry, transferred it to the desiccator with a lid that was partially covering it, in order to cool it down. Reweighed the dish and the dehydrated sample this is the final weight W2.

Moisture (%) = (W1-W2) / W1 x 100

W1 weight (g) of sample before drying,

W2 weight (g) of dried sample

Fig 3:Spirulina samples being dried to remove moisture in hot air oven

3.2 Chemical Tests

Food chemical tests are utilized to quantify the nutritional composition of food items, including the quantities of vitamins, minerals, and macronutrients, in order to verify that the food product meets particular nutritional standards**.** Food and beverage samples are subjected to chemical analysis to confirm that they are safe to consume, meet quality standards, and maintain their integrity.

3.2.1 Ash Content

Ash content analysis is a relatively simple and technique used for evaluating the mineral composition of food items. It can offer useful information regarding the nutritional value of edible products. This method is employed to ascertain the proportion of vital nutrients, such as calcium, phosphorus, potassium, magnesium, iron, and zinc, which are necessary minerals that perform crucial tasks in many physiological processes within the human body. The test technique was derived from the ash content analysis described in the

To acquire the weight of the sample (Ws), 2 grams of each dried sample (with moisture removed from a moisture content test) were well mixed and precisely weighed. The desiccated specimen was carefully positioned into a porcelain crucible that had been previously weighed and dried. The weight of the crucible, denoted as W1, was recorded. The sample was heated to 600°C in a muffle furnace for 2 hours, then cooled to ambient temperature in a desiccator and weighed to determine W2, ensuring all organic substances were burned. Ash content in food samples is calculated using the formula ash percentage = (W2 - W1) / Ws, where W2 represents crucible weight, W1 represents empty crucible weight, and Ws represents dry sample weight.
Ash Content (%) = Wt of ash/Wt of sample x 100

Fig 4: Displays the charred residue of the samples after undergoing treatment in the muffle furnace

3.2.2 Determination of Polyphenols

Phenolics are present in all types of food, although they are typically found in very little quantities. Phenolic-rich foods that are worth mentioning include coffee and tea, chocolate, fruits and their derivatives, some oils, spices, and select whole grains.

While initially developed for analyzing wines and grapes, these methods can be used for analyzing different types of food. The presence of polyphenols in food plays a vital role in maintaining stability due to the association between the amount of total polyphenols and the ability to resist oxidation over time. Polyphenols exert a protective effect on both food and human cells by counteracting the detrimental impact of free radicals, hence exhibiting anti-aging properties. Phenolic chemicals found in food and microalgae are associated with advantageous biological effects such as antioxidant capacity, antibacterial properties, and immunomodulatory activities. (Cichonski & Chrzanowski, 2022).

3.2.3 Total Phenolic Content

The Folin-Ciocalteu (FC) assay is a frequently used colorimetric technique for quantifying the overall phenolic content in food items. The assay operates based on the mechanism of phenolic chemicals reducing the FC reagent, resulting in the formation of a blue-colored complex that absorbs light at a wavelength of 760 nm (Singleton & Rossi, 1965). The research methodology employed in the present research was adapted from the methods outlined by Burapan et al. (2020), Waterhouse

(2002), and Singleton & Rossi (1965). Take 2g test sample in 15ml test tube then add 5ml (methanol 80%). Keep the sample in ultrasonic bath for 15min. Incubate for 2hrs. After incubation period, take/collect the upper phase of methanolic extract in caped Eppendorf. A 1 mg/mL gallic acid (GA) stock solution was prepared by dissolving 1 g of gallic acid in 1000 mL of distilled water. Subsequently, a series of 5 dilutions were made, ranging from concentrations of 0.0625-1 mg/mL. Similarly, a solution of sodium carbonate with a concentration of 7.5% was prepared by dissolving 7.5 grams of the powder in 100 milliliters of distilled water. The test tubes for the GA and ethanolic sample extracts were made by sequentially adding 3.16 mL of distilled water, 0.2 mL of FC Reagent, and 0.6 mL of the 7.5% sodium carbonate solution. Next, 40µL of the GA dilutions and sample extracts were added to the test tube, and the tube was vigorously mixed using a vortex after each addition.

After covering the mixtures with aluminum foil, they were left to dry out at room temperature for two hours while being completely dark. Using a spectrophotometer, the absorbance of the resultant greenish hue was then measured at 760 nm, and a standard curve was constructed. Using the GA standard curve, the phenolic content was calculated and expressed as a percentage of the initial sample weight.

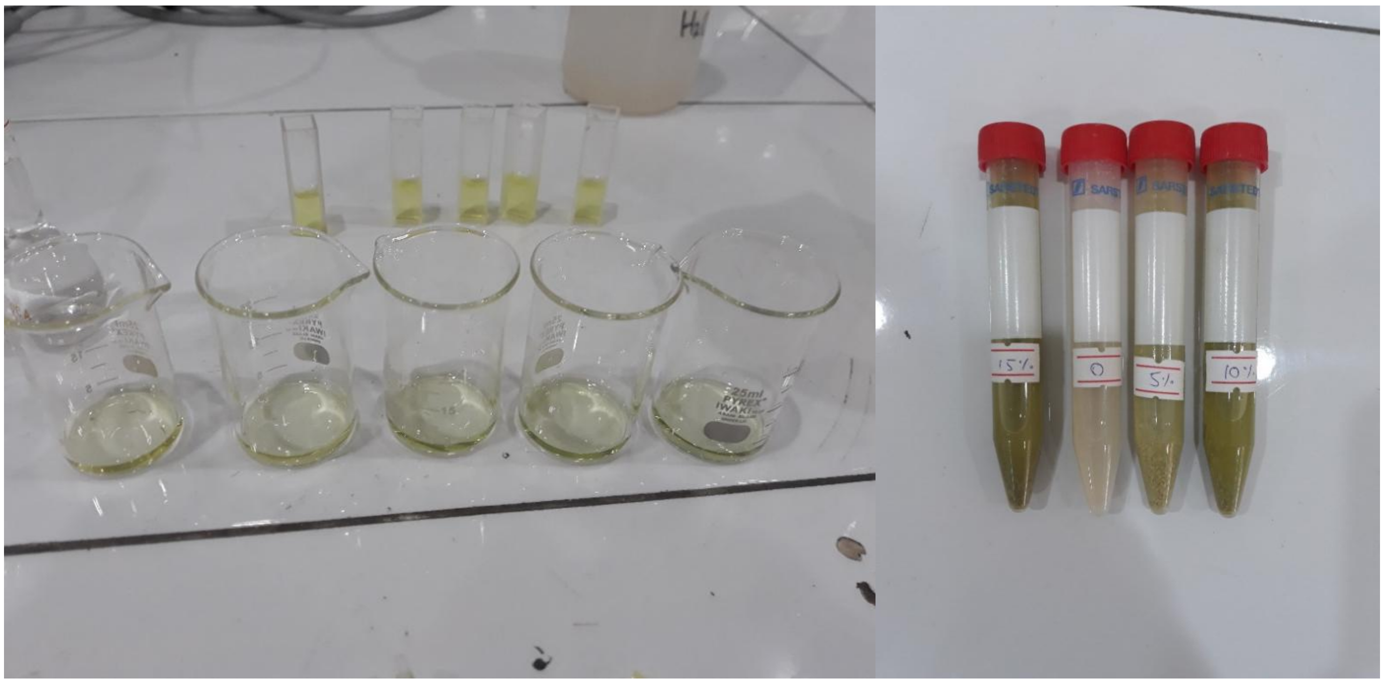

Fig 5: Phenolic Content Test

3.2.4 Crude Fat Test

Fat is essential for energy, cell function, and vitamin absorption. Total fat, as per nutrition facts labels, consists of saturated, polyunsaturated, and monounsaturated fats per serving. Accurate fat content can help reduce obesity and heart disease. Crude fat, a mixture of fat-soluble compounds, Crude fat analysis is a method used to measure the overall fat content in food samples. This is significant because it helps assess the nutritional value of the tested goods, as fats and fatty acids are important components of the human diet.

For analysis 5 grams of sample was measured (Ws) then the extracted substance was carefully placed inside a filter paper thimble, ensuring that no leakage occurred. The sample was acquired through the utilization of a Soxhlet apparatus and subjected to extraction using an organic solvent having a low boiling point. The extraction method involved the use of petroleum ether, which was carried out at a temperature of 60ºC for several hours. The extraction was considered effective when complete removal of fat was achieved, as shown by the solvent being completely transparent. After an hour of evaporation in a hot air oven, the extracted fat was placed in a desiccator to extract the solvent. After that, the residual fat was measured. Empty flask weight W1 (g)

Weight of extracted fat and flask W2 (g).

Sample weight = S

formula used

(W2 – W1) x 100/S equals crude fat (%).

of AOAC International. AOAC International, Gaithersburg, MD, USA.CRUDE FAT TEST:

Fig 6: Crude Fat Test. The samples obtained using a Soxhlet equipment

3.2.5 Test for Crude Protein Content

Dumas Combustion Method Dumas combustion method, creative dry chemistry, simple application. Approved formally for the purpose of determining grain nitrogen and protein content. NDA 702 Dumas combustion apparatus is ideal choice with high throughput, an entirely automated system taking just 3-4 minutes per analysis having great productivity and non-stop performance.

The process starts with a combustion furnace (CF) to burn the material, obtaining elemental components in an oxygen atmosphere subsequently via oxidation and reduction tubes. After the combustion, a first physical trap (WT1) removes water, then a second chemical one (WT2). Between the two, the elemental elements flowed via an RF reduction furnace. Without reference gas, the auto-regenerative CO2 absorbers (CO2) allow pass just the elemental nitrogen identified by Thermal Conductivity Detector (TCD). PC controls the NDA 702 using a DUMASoftTM.

From all pasta sample, grind finely using a grinder, collect 100 grams, put 1 gram (100 mg) of sample straight into the tin foil with a spatula. Close the tin foil, then load a capsule into the autosampler NDA 702 for combustion using the operating manual to verify that the following values:

Combustion reactor temperature: 1030 °C

Reactor for reduction of temperature: 650 °C

MFC1 Carrier Gas Flow Rate: 190 ml/min He

MFC2 Carrier Gas: 220 ml/min He

6.25 is the protein factor.

O_2 flow: 400 ml/min.

1.6 ml/mg O_2 factor

Fig 7: Crude Protein Test

3.2 Scanning Electron Microscopy (SEM)

To assess the validity and reliability of measurements SEM was used.The operational mechanism of a scanning electron microscope (SEM) is based on an electron beam striking the sample, this bean dislodges electrons from the sample. The energy of these ejected electrons is determined by the energy released by the atomic shell and can be measured using detection techniques. SEM images are generated by scanning electrons across the sample's surface, exploiting variations in contrast based on factors like surface topography, magnetism, atomic number, conductivity, and crystallographic orientation. SEM has been used to study pasta product processing (Fardet et al,. 1998).

3.3.1 Sample Preparation

Scanning electron microscopy was used to examine the particle morphology of the desired powdered samples (SEM JSM- 6490 JOEL). In order to prepare specimens for SEM inspection, the structures as indicated in each analysis must be stabilized. The sample was mounted on a sample stub using carbon conducting tape, and it was then sputtered with gold in an Auto Fine Coater (JFC – 1600, JEOL) for 30 seconds. After that, the samples' stubs were put into the SEM's sample chamber. Following the conventional processes for machine evacuation, the samples were examined to determine the particle's morphology. The accelerating voltage and sample distance from the electron cannon tip were set at 10 mm and 15 kV, respectively (Golding et al., 2016).

The parameters of our nanomaterial are as follows: diameter of a fiber, condensed or not, texture, pore size, shrinkage, distribution, e.g. length, thickness, particles size on scale bar of 100um; cross-section to see the thickness of layers, surface layer morphology, whether the dimensions of the fibers are hollow or compact (solid), smooth, rough, or something in between; and finally, to characterize the surface morphology of our nanomaterial to see its nature.

3.4 Statistical Analysis

To do statistical analysis on the experimental data, the Python "scipy.stats" module was utilized. All the results are presented as average ± 1standard deviation. Variance analysis (one-way ANOVA) was conducted for each test (protein, ash, fat, phenolic, and moisture) comparing the control sample with each test sample (5%, 10%, 15%). ANOVA was chosen to assess whether the observed differences in means were statistically significant or could be attributed to random variation. Determined the F-critical value for each test based on the degrees of freedom F-critical value serves as a threshold. If the F-statistic exceeds this value, the null hypothesis is rejected .. Calculated the F-statistic for each comparison, ie ratio of the variance between the group mean values to the variance within the groups. A higher F-statistic indicates a greater difference between the group mean values relative to within-group variance. The p-value gives a precise probability, which helps quantify the strength of the evidence against the null hypothesis. Used as additional tool to ensure that the decision made based on the F-statistic is robust. Significance level of $p < 0.05$ was kept as threshold.

` CHAPTER 4: RESULTS AND DISCUSSION

Table 2: Results and Standard Deviation

Sr. No	Treatment	Crude Protein (g/100 g)	Ash (g/100 g)	Crude Fat (g/100 g)	Phenolic (mg GAE/g DW)	Moisture (MC%)
1	**Control 0%**	**11.8 ± 0.39**	**1.0 ± 0.083**	**1.8 ± 0.14**	**0.0 ± 0.02**	**1.1 ± 0.02**
2	**5%**	**12.3 ± 0.75**	**1.4 ± 0.084**	**2.5 ± 0.27**	**0.1 ± 0.03**	**1.1 ± 0.02**
3	**10%**	**13.2 ± 0.16**	**1.5 ± 0.034**	**2.6 ± 0.21**	**0.5 ± 0.11**	**1.0 ± 0.02**
4	**15%**	**13.5 ± 0.13**	**1.9 ± 0.047**	**2.7 ± 0.11**	**0.7 ± 0.15**	**1.0 ± 0.02**

Table 3: Significance based on F-Static and p-value

Comparison	**F-statistic**	**p-value**	**F-critical**	**Significant**
Protein - Control vs 5%	1.51599	0.285	7.708	**No**
Protein - Control vs 10%	20.1506	0.010	7.708	**Yes**
Protein - Control vs 15%	37.7358	0.003	7.708	**Yes**
Ash - Control vs 5%	31.307	0.005	7.708	**Yes**
Ash - Control vs 10%	61.932	0.0014	7.708	**Yes**
Ash - Control vs 15%	166.816	0.00020	7.708	**Yes**
Fat - Control vs 5%	28.4137	0.0059	7.708	**Yes**
Fat - Control vs 10%	66.4534	0.0012	7.708	**Yes**
Fat - Control vs 15%	13.4993	0.021	7.708	**Yes**
Phenolic - Control vs 5%	4.73	0.095	7.708	**No**
Phenolic - Control vs 10%	89.3055	0.00069	7.708	**Yes**
Phenolic - Control vs 15%	254.393	9.03324E-05	7.708	**Yes**
Moisture - Control vs 5%	3.04054	0.156	7.708	**No**
Moisture - Control vs 10%	7.37705	0.0532	7.708	**No**
Moisture - Control vs 15%	34.5156	0.00419343	7.70865	**Yes**

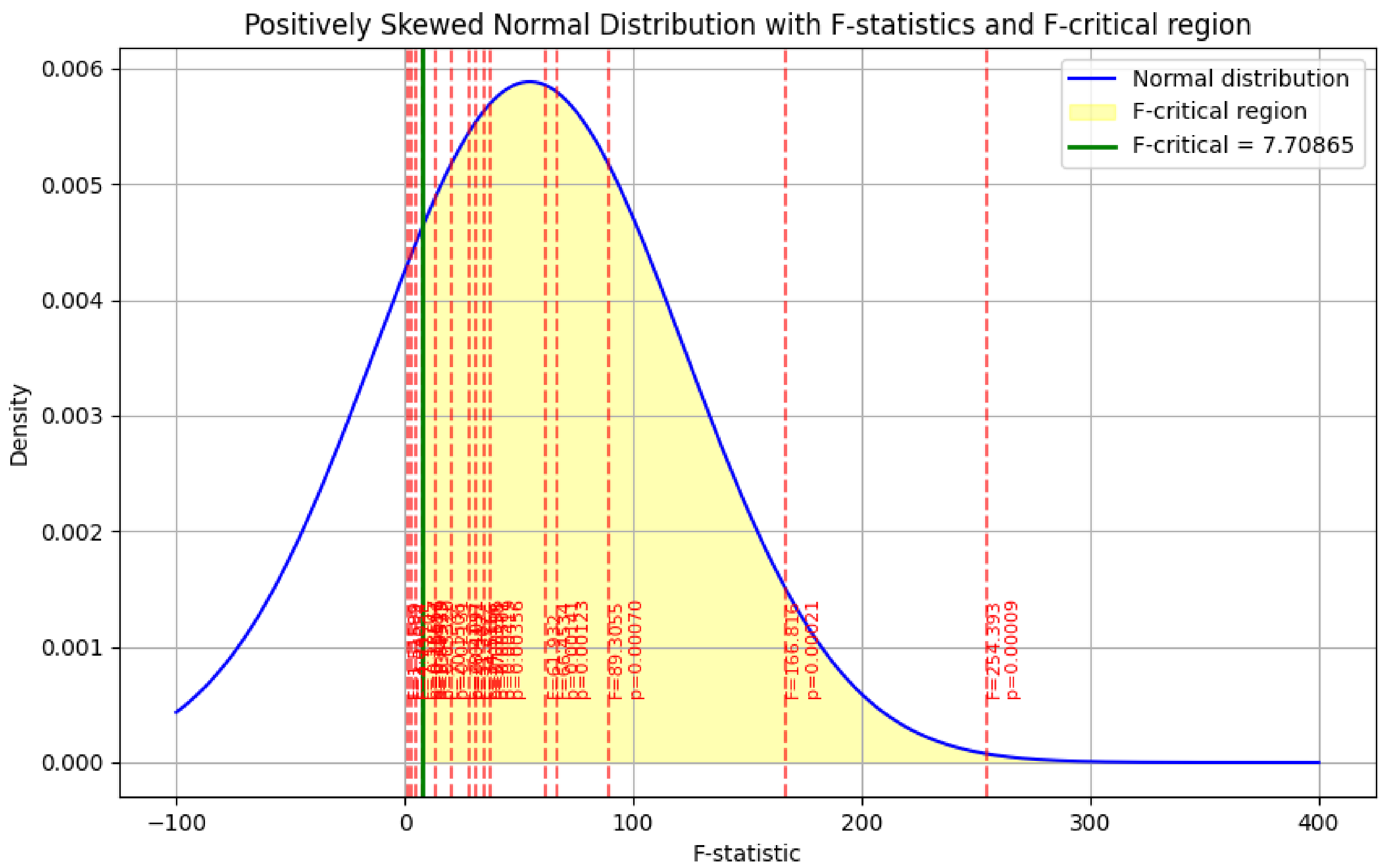

Fig 8: Positively Skewed Distribution of F-Static, with F critical region supplemented by p-value

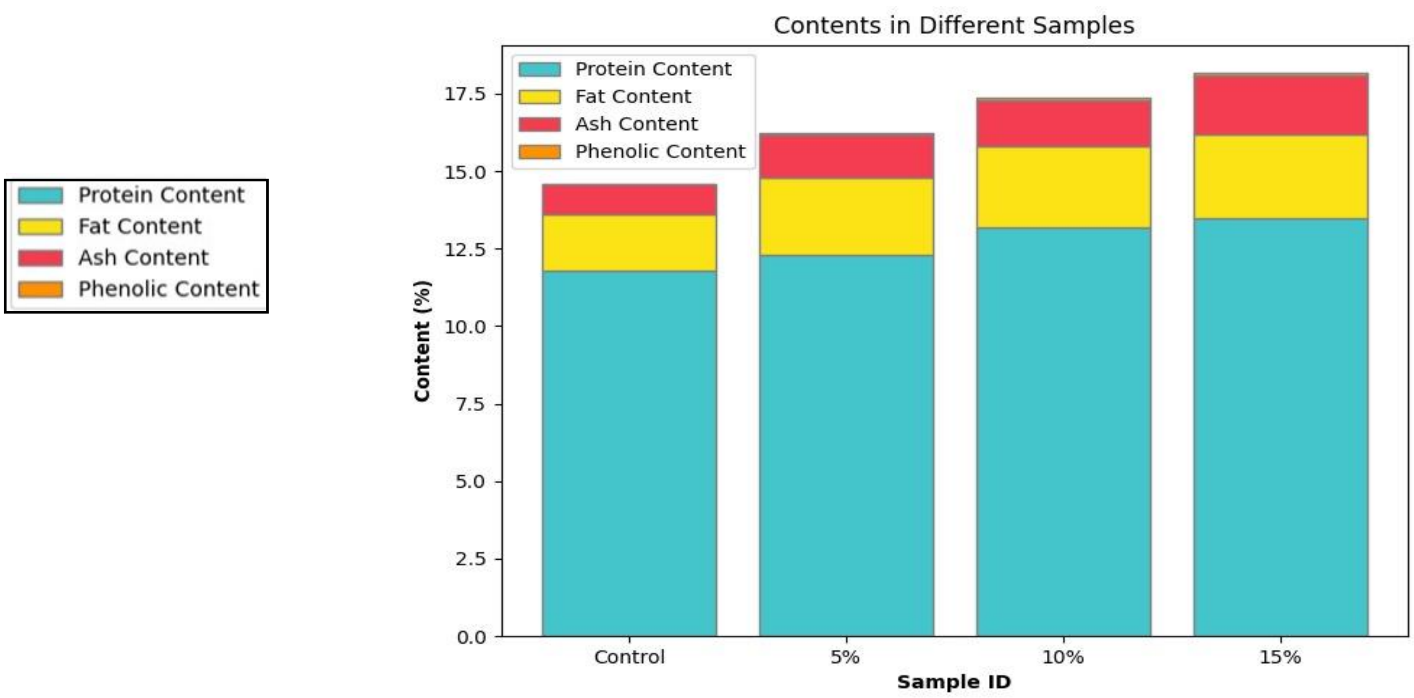

Fig 9: Sample Content

The values for analysis are presented as the mean 1 ± standard deviation (S.D) and carried out in triplicate. (Fig 1). The values in each row indicate a statistically significant difference ($p < 0.05$). The values in this table correspond to the three formulations of Spirulina Arthrospira 5%,10%,15% in Rice Flour (RF) and Quinoa flour (QF) in comparison with the control sample which does not contain Spirulina.

The data provided for the spirulina supplementation at 5%, 10%, and 15% levels in gluten-free quinoa and rice flour for pasta making can be interpreted in terms of changes in protein, ash, fat, phenolic content, and moisture compared to the control (no spirulina supplementation).

4.1 Effect of Spirulina on Moisture Content

The moisture content decreases slightly with increasing spirulina, from 1.1% in the control to 1.0% at 15% spirulina. None of the spirulina supplementation levels show statistically significant differences in moisture content ($p>0.05$ for all).

The results show Control sample (without spirulina) has the highest moisture content at 1.1%. At 5% of Spirulina Moisture content decreases slightly to 1.1%, while at 10% Spirulina there was a further reduction to 1.0% was seen. At 15% Spirulina there is a lowest moisture content observed at 1.0%. Moisture content slightly decreases with increasing spirulina levels. This may affect the texture and shelf-life of the pasta, potentially making it drier and possibly more brittle. In an investigation of Spirulina enhanced snack bars, Lucas et al. (2020) examined how moisture levels can serve as indicators of food shelf life. Higher moisture content is associated with elevated levels of microbial growth and food degradation. The manufactured pasta corresponded with the FDA's (Food and Drug Administration) moisture content standards. It has long been acknowledged that the suggested figure of 3% is a suitable gray region for pasta products. Pasta moisture loss of no more than 3% was formerly accepted by the FDA and NIST.

There is a clear trend indicating that 5% and 10% Treatments: No significant difference from the control ($p > 0.05$).5% and 10% Treatments:15% Treatment: Significant difference from the control ($p < 0.05$), indicating that the treatment has a statistically significant effect on moisture content.

4.2 Effect of Spirulina on Protein Content

The crude protein content increases with increasing levels of spirulina supplementation, from 11.8 g/100 g in the control to 13.5 g/100 g. At 5% spirulina supplementation shows no significant difference ($p=0.285670$), at 10% and 15% spirulina supplementation both show statistically significant increases in protein content ($p=0.010915$ and $p=0.003561$ respectively). The data provided shows the impact of Spirulina supplementation on the protein content of quinoa and rice flour gluten-free pasta. There is a noticeable trend, where increasing the Spirulina content generally increases the overall protein content of the gluten-free pasta. At 5% Treatment: No significant difference from the control ($p > 0.05$). At 10% and 15% Treatments: Significant differences from the control ($p < 0.05$), indicating that the treatments have a statistically significant effect on protein content. In a study conducted by (Hussein A., et al 2021) on Spirulina pasta showed the same results that the Pasta's protein level increased with increasing Spirulina concentration its rheological parameters, color, and cooking quality raised as well, while dough stability decreased. According to sensory evaluation 5% of Spirulina supplementation of pasta samples was acceptable in this study. Spirulina-enriched pasta is a rich source of protein and antioxidants. The enrichment of pasta caused a reduction in sensory scores because of its earthy flavor with an increase in the addition level.

4.3 Effect of Spirulina on Ash Content

The ash content also increases with higher spirulina levels, from 1.03 g/100 g in the control to 1.90 g/100 g at 15% spirulina. - All spirulina supplementation levels (5%, 10%, and 15%) show statistically significant increases in ash content ($p<0.005$ for all).

The control sample has an ash content of 1.03%, serving as the baseline for comparison. Ash content, which indicates mineral content, initially increases to 1.4%, at 5% supplementation, then significantly increases at 10% and 15% levels to,1.5% and 1.9% respectively. This suggests that higher spirulina levels contribute more minerals to the pasta.

The ash content of 1.03% in control sample is due to the presence of Quinoa as a potential addition to too in a gluten free pasta. The highest ash content of 1.90% is observed, indicating a significant increase in mineral content with the highest s All Treatments (5%, 10%, 15%): Significant differences from the control ($p < 0.05$), indicating that the treatments have a statistically significant effect on ash content spirulina supplementation. This suggests that spirulina with Quinoa contributes more to the mineral profile of the pasta.

4.4 Effect of Spirulina on Fat Content

All spirulina supplementation levels (5%, 10%, and 15%) show statistically significant increases in fat content ($p<0.05$ for all). The crude fat content increases from 1.8 g/100 g in the control to 2.7 g/100 g at 15% spirulina supplementation.

The control pasta (without spirulina) had a fat content of 1.8 ± 0.14 g/100g.Pasta with 5% spirulina supplementation had a fat content of 2.5 ± 0.21 g/100g. Pasta with 10% spirulina supplementation had a fat content of 2.6 ± 0.11 g/100g, while the pasta with 15% spirulina supplementation had a fat content of 2.7 ± 0.27 g/100g.Fat content increases with spirulina supplementation. Spirulina contains essential fatty acids, which likely contribute to the higher fat content observed in the supplemented pasta. Based on the results it appears that the addition of spirulina to quinoa and rice flour pasta had a significant impact on the fat content of the final product. All Treatments (5%, 10%, 15%): Significant differences from the control ($p < 0.05$), indicating that the treatments have a statistically significant effect on fat content.

4.5 Effect of Spirulina on Phenolic Content

The phenolic content, measured in mg GAE/g DW, increases significantly with higher spirulina levels, going from 0.0 in the control to 0.7 at 15% spirulina. At 5% spirulina, supplementation shows no significant difference in phenolic content ($p=0.095291$),10% and 15% spirulina supplementation both show statistically significant increases in phenolic content ($p<0.001$ for both).

Control Sample: The control sample has the lowest TPC, with a value of 0.0 mg GAE/g DW. This indicates a relatively no phenolic content compared to the spirulina-supplemented samples. The addition of 5% spirulina increases the TPC to 0.12 mg GAE/g DW, suggesting that even a small quantity of spirulina significantly enhances the phenol. With 10% spirulina, the TPC rises substantially to 0.5 mg GAE/g DW. This indicates a strong correlation between spirulina concentration and phenolic content. The highest spirulina concentration (15%) results in the highest TPC of 0.7 mg GAE/g DW, reflecting a significant enhancement in phenolic content due to spirulina supplementation. The Total Phenolic Content increases with the addition of spirulina in gluten-free pasta. There is a clear trend showing that higher spirulina concentrations lead to higher phenolic content, enhancing the antioxidant potential of the pasta. With 5% Treatment: No significant difference from the control ($p > 0.05$). With 10% and 15% Treatments: Significant differences from the control ($p < 0.05$), indicating that the treatments have a statistically significant effect on phenolic content. These findings indicate that spirulina supplementation not only increases the nutritional constituents of this pasta but also significantly boosts its phenolic content, potentially offering greater health benefits. But the addition of Quinoa to a rice flour with spirulina supplementation is a good source of saponins and phenolic compounds. Quinoa is particularly rich in histidine and lysine, essential amino acids deficient in most cereals.

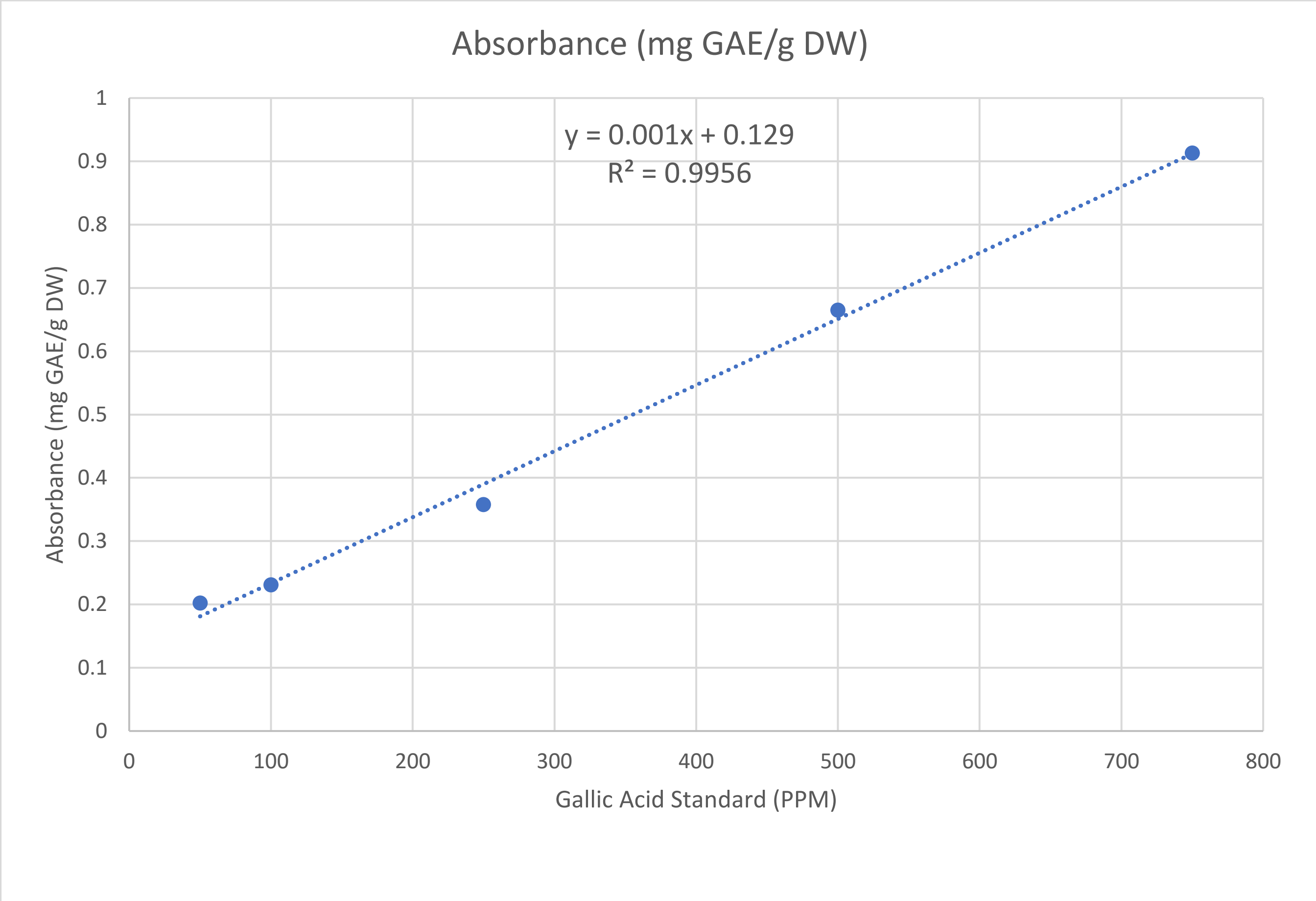

Fig 10: Absorbance Calibration Curve Analysis

The graph shows the relationship between the absorbance and the concentration of Gallic Acid Standard (PPM). The equation of the trend line is: [$y = 0.001x + 0.129$]; where (y) is the absorbance and (x) is the concentration of the standard in PPM. The high R^2 value of 0.9956 indicates a strong linear relationship.

Sample Analysis

Using the trend line equation, the absorbance values of the samples can be interpreted to determine their TPC in mg Gallic Acid Equivalents (GAE) per gram Dry Weight (DW).

Table 4 : Absorbance values for Gallic Acid Standard (PPM)

Standards (PPM)	Absorbance (mg GAE/g DW)
50	0.2018
100	0.2309
250	0.3576
500	0.6645
750	0.9128

Table 5 : TPC in mg Gallic Acid per Gram Dry Weight

	SAMPLE NAME	AB	TREND	RESULTS TPC (MG GAE/G DW)
1	Control	0.1447	0.0157	0.0785
2	5%	0.1548	0.0258	0.129
3	10%	0.2424	0.1134	0.567
4	15%	0.2705	0.1415	0.7075

Overall, the data shows that adding spirulina to rice and quinoa gluten-free pasta increases the nutritional profile of the pasta, with higher protein, ash, fat, and phenolic content as the spirulina supplementation level is increased from 5% to 15%. The moisture content decreases slightly but remains low across all treatment. This could make the pasta more nutritious but also alter its physical properties.

The ANOVA results show that the higher percentage treatments (10% and 15%) generally have a significant impact on the protein, ash, fat, and phenolic content compared to the control sample. The 5% treatment has a significant effect on ash and fat content but not on protein, phenolic, or moisture content. The 15% treatment significantly affects moisture content, whereas the 5% and 10% treatments do not.

4.6 Scanning Electron Microscope Results

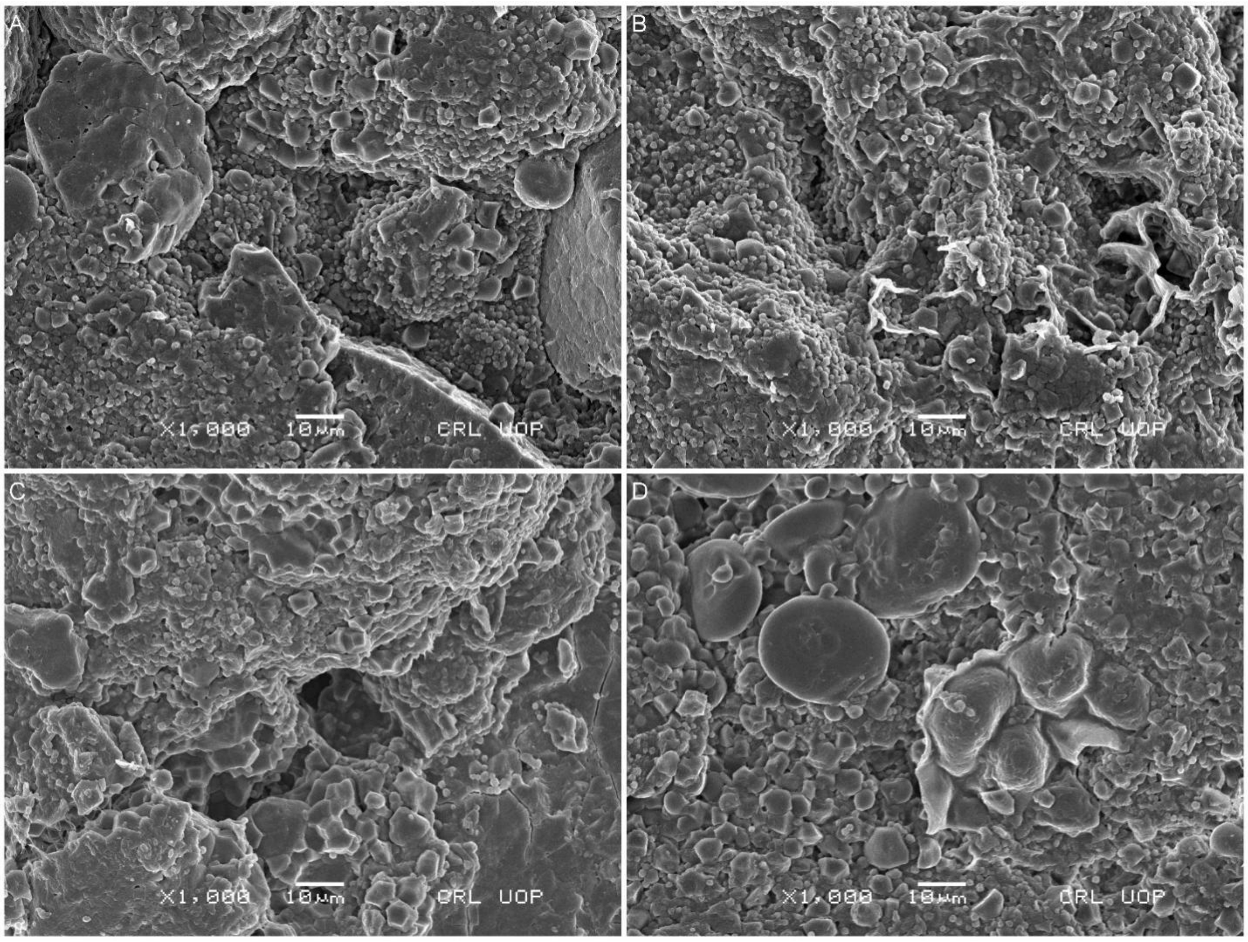

Fig 11: SEM Magnification X1000 (10 Microns)

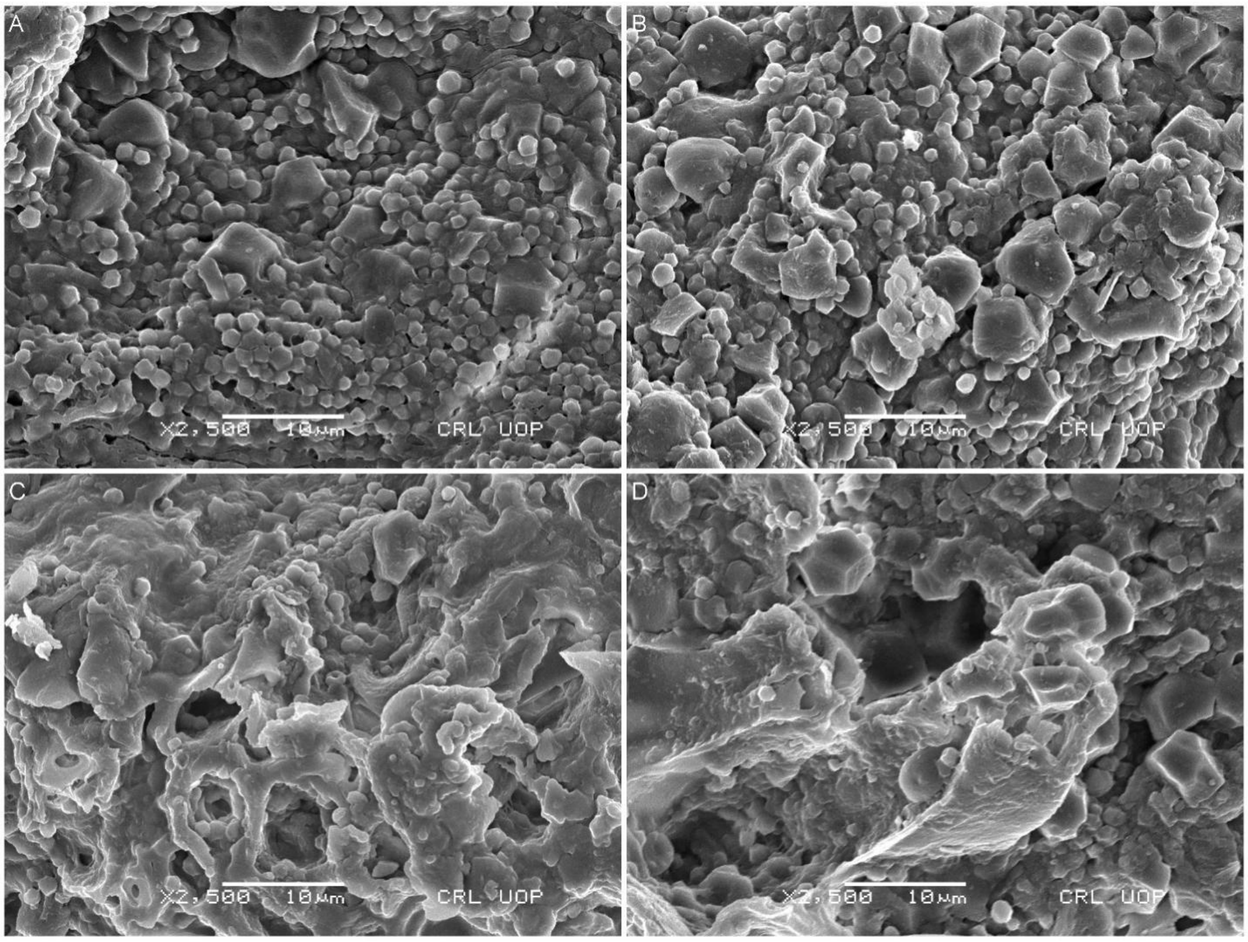

Fig 12: SEM Magnification X 2500

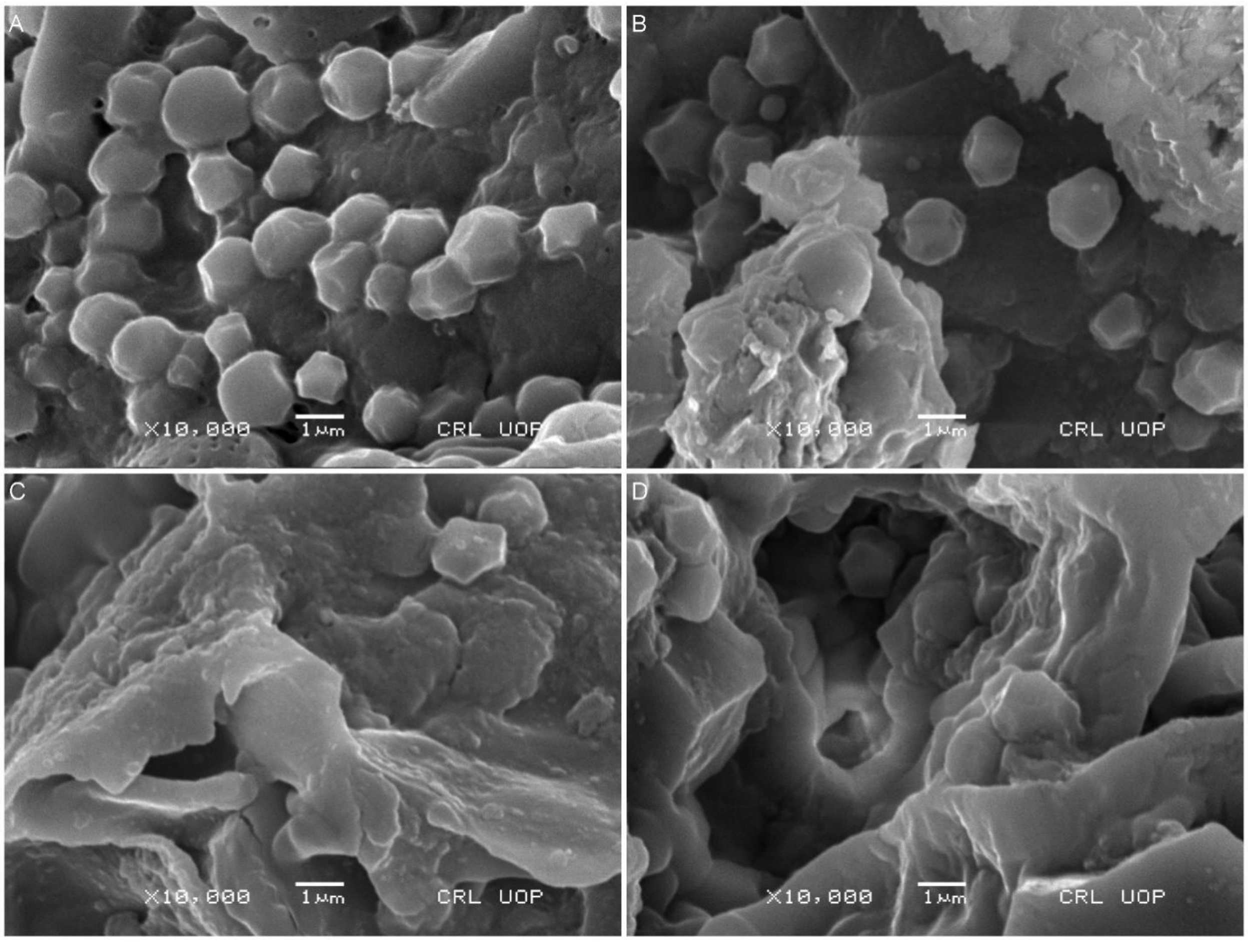

Fig 13: SEM Magnification X10000 (1 UM)

RF+ QF	=	control sample A
RF+QF+SP 5%	=	sample B
RF+QF+SP 10%	=	sample C

RF+QF+SP 15% = sample D

These SEM images displayed the morphological characteristics of gluten-free pasta made from rice and quinoa, supplemented with varying percentages of spirulina (5%, 10%, 15%) compared to a control sample.

In fig (4) the scanning electron microscope (SEM) image (1um) at 10,000 magnification shows the microstructure of gluten-free pasta surface structures containing rice, quinoa, and spirulina. The detailed analysis is based on three-dimensional characteristics of appearance size and shape of Rice, Quinoa, and Spirulina Molecules.

1. Rice Starch Granules:

- Size of the rice starch granules typically ranges from 3 to 8 micrometers in diameter. Shape usually appears as small, smooth, and polygonal or round granules. Image A likely represents rice starch granules, showing uniform, small, and round/polygonal particles (Lindeboom et al., 2004).

2. Quinoa Starch Granules:

- Size of the Quinoa starch granules are generally between 1 and 3 micrometers. Its shape is small and oval or spherical. Image B appears to show quinoa starch granules, which are smaller and more varied in shape compared to rice starch.

3. Spirulina Particles:

- Size of Spirulina particles can vary greatly but are generally very small (less than 1 micrometer). Its Shape being a type of cyanobacteria, has a filamentous or irregular shape when dried and powdered. Image C and D may represent spirulina particles, showing irregular and fragmented shapes interspersed within the matrix.

Image A: Control Sample

- **Morphology**: The control sample, which has no spirulina, shows a relatively homogeneous structure with fewer voids and a denser matrix. The surface appears compact with fewer irregularities and uniform structure with tightly packed granules indicating a well-formed matrix. The control sample exhibits a relatively smooth and a densely packed structure with uniformly sized rice and quinoa granule.

- **Protein-Starch Matrix:** The control sample exhibits a relatively smooth and compact structure. The matrix shows strong cohesion, with proteins and starches interacting effectively to form a continuous network. The granules are densely packed with fewer voids and irregularities., The protein-starch matrix seems to be continuous and well-formed, indicating good interaction between the rice and quinoa proteins and starches This results in a stable and compact network.

Image B: Pasta with 5% Spirulina

- **Morphology**: The addition of 5% spirulina introduces noticeable changes. The matrix appears more fibrous and less dense compared to the control. There are more visible voids and a rougher texture. The granules are somewhat larger and less uniformly packed, with minor increases in void spaces.

Protein-Starch Matrix: The protein-starch interaction seems less cohesive, possibly due to the introduction of spirulina, which might disrupt the original matrix structure. The fibrous nature could be attributed to the spirulina content. The spirulina seems to cause a slight weakening of the interactions between proteins and starches, but the overall matrix remains largely intact. The network is still continuous but less compact than the control.

Image C: Pasta with 10% Spirulina

- **Morphology**: With 10% spirulina, the structure becomes even more disrupted. Larger voids and a more porous texture are evident. The matrix appears less compact. The structure becomes more porous with 10% spirulina. There are noticeable voids and a rougher texture. The granules appear less densely packed. The granules are more irregular in size and shape, and the overall packing density decreases further, leading to increased porosity.

- **Protein-Starch Matrix:** The protein-starch network is further weakened, indicating that higher spirulina content significantly affects the matrix formation. There is a more pronounced separation between different components. The protein-starch matrix shows more significant disruptions. The interaction between the components is weaker, leading to a less cohesive network. The protein-starch matrix exhibits more significant disruptions. The interactions between the proteins and starches are weakened, resulting in a less cohesive and more fragmented network.

Image D: Pasta with 15% Spirulina

- **Morphology**: At 15% spirulina, the morphology shows the most significant changes. The structure is highly porous with many large voids and a very rough texture. The matrix appears highly irregular and broken. At 15% spirulina, the structure is highly porous and irregular. Large voids and a highly disrupted texture are evident.

These SEM images displayed the morphological characteristics of gluten-free pasta made from rice and quinoa, supplemented with varying percentages of spirulina (5%, 10%, 15%) compared to a control sample.

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3. Spirulina Particles: The Size of Spirulina particles can vary greatly but are generally very small (less than 1 micrometer). Its Shape being a type of cyanobacteria, has a filamentous or irregular shape when dried and powdered. Image C and D may represent spirulina particles, showing irregular and fragmented shapes interspersed within the matrix.

Protein-Starch Matrix:

In Control sample it is seen that there is a dense, compact, and continuous matrix, indicating good protein-starch interaction. With 5% Spirulina there is an introduction of fibrous structure and slight disruption in the matrix. With 10% Spirulina Increased porosity and more significant disruption of the protein-starch matrix is observed. 15% Spirulina a highly porous, irregular structure with a severely compromised matrix. The continuity of the matrix is heavily disrupted, indicating poor interaction between the rice, quinoa proteins, starches, and the high spirulina content. The matrix is severely compromised, with a fragmented and discontinuous network. The high spirulina content significantly disrupts the interaction between proteins and starches, leading to a weak and irregular matrix. So, it was also qualitatively observed as an unstable and weaker final product. The addition of spirulina affects the structural integrity and morphology of the gluten-free pasta, with higher spirulina concentrations leading to more significant disruptions in the protein-starch matrix. This suggests that while spirulina can be beneficial for nutritional content, its impact on the structural properties of gluten-free pasta needs careful consideration.

Functional Groups in a Protein-Starch Matrix

The protein-starch matrix in gluten-free pasta is composed of various functional groups from the proteins and starches. Functional Groups in Proteins are amino groups (-NH2), carboxyl groups (-COOH), Amide bonds (-CONH-)

Proteins contribute to the structural matrix and interact with starch molecules through hydrogen bonding and electrostatic interactions. When spirulina is added to gluten-free Quinoa and Rice flour it makes a protein matrix with scattered starch granules. Functional Groups in Starch are Hydroxyl groups (-OH) and Glycosidic linkages (-O-) Starch granules provide the bulk of the carbohydrate content and interact with proteins to form the matrix. At higher spirulina concentrations there is more significant disruptions in the protein-starch matrix that is because the complicated structural network of gluten in wheat-based products mostly determines the quality of the pasta and in the absence of gluten proteins it becomes difficult to form the protein network that occurs in gluten-free pasta. When the percentage of spirulina was increased the percentage of quinoa decreased which was acting as the binding material for the pasta and is equally responsible for the structural integrity of pasta.

Interaction in the Protein-Starch Matrix

Hydrogen Bonding: The hydroxyl groups of starch interact with the amino and carboxyl groups of proteins, forming a network of hydrogen bonds that contribute to the structural integrity of the pasta. Hydrophobic Interactions are non-polar side chains of proteins may interact with hydrophobic regions of other proteins or the non-polar parts of starch molecules. Electrostatic Interactions are charged side chains of amino acids in proteins can form ionic bonds with oppositely charged groups in other proteins or with charged regions of starch molecules.

Starch is a complex combination of two forms of α-glucans, namely amylose and amylopectin. It is stored in plants as granules, which vary in size and shape depending on the species. The granules have a diameter that varies from less than 1µm to over 100µm. The adhesive properties and usefulness of rice are mostly governed by the starch component. The process of cooking a raw rice grain involves several transformations, including glass transition, swelling, gelatinization, pasting, leaching of amylose, and degradation of the starch granules. These transformations are responsible for achieving the desirable textural features in the cooked grain (Fitzgerald, 2004).

CONCLUSION

The color of the pasta had been significantly affected by the addition of spirulina. Considering the use of sustainable and protein-rich resources, pasta has great potential for enrichment with spirulina (Arthrospira platensis). This is owing to its simple manufacturing technique, affordability, nutritional value, and widespread acceptance.

Food pasta production is a mature technological process, widely consumed due to its versatility, easy storage and cooking. It is made by combining wheat flour or durum wheat flours with potable water, followed by kneading, molding, and drying the mixture. Pasta is known for its high carbohydrate content and low levels of minerals, vitamins, bioactive compounds, and essential amino acids such as lysine and methionine for human consumption. Researchers have been working on increasing the protein content of pasta by integrating plant-based raw materials in order to satisfy consumer demands for quality. Quinoa an important food crop, is recognized by FAO for its high nutritional and functional quality, making it an ideal candidate for extruded pasta, and Spirulina a cyanobacteria is used as an alternative ingredient in pasta industry. Therefore, it is important to focus on the composition and functionality of the raw material. This project aims to evaluate the effect of total substitution of wheat flour with the rice and quinoa flour on starch and protein content, physicochemical properties.

In the results there is a clear trend indicating that increasing spirulina supplementation in quinoa and rice flour pasta correlates with a reduction in moisture content. This could be due to the hygroscopic properties of spirulina, which possibly affect the water retaining capability of the pasta matrix. The pasta processing conditions could impact the relationship between spirulina content and moisture. This is an important consideration that's worth exploring further.

On industrial scale production pasta drying temperature and extrusion parameters can significantly influence the final moisture content, and these factors may interact with the presence of spirulina in complex ways. Higher drying temperatures could accentuate the hygroscopic effects of spirulina, leading to greater moisture loss as the spirulina competes more aggressively for available water, it may also disrupt the pasta matrix structure, counteracting the spirulina's moisture-binding influence. An optimal drying temperature range may exist that balances this competing effect. More intense extrusion could disrupt the spirulina's water-binding capacity or alter its distribution within the pasta. It would be valuable to systematically investigate the interactions between spirulina content, drying temperature, and extrusion variables to map out how these processing factors influence the final moisture profile of the pasta (Lindeboom et al,.2004).

These structural changes to the pasta matrix may alter how it interacts with the spirulina components, affecting moisture content. The structural changes in the pasta matrix were examined using techniques like microscopy. Gaining a deeper understanding of these processing-composition interactions would provide important insights for optimizing spirulina-fortified pasta formulations and manufacturing processes.

This variability should be considered when interpreting the results, as it may affect the reliability of the observed trend. The degree of reduction correlates with the percentage of Spirulina added. While there are slight variations, the overall trend suggests that as more Spirulina is added, the protein content increases. This information can help in optimizing the formulation based on the desired nutritional profile and benefits from Spirulina.

The ash content analysis reveals that increasing spirulina concentration in gluten-free pasta affects its mineral content. While a 5% spirulina addition reduces the ash content, higher concentrations (10% and 15%) increase it, with the 15% spirulina sample showing the highest mineral content. This suggests that the spirulina-quinoa combination positively impacts the mineral composition of the pasta.

The results indicate that the incorporation of spirulina into the pasta formulation had a considerable impact on the final fat content, with the highest fat content observed at the 10% spirulina level. Spirulina has a naturally high fat content, which can vary from 4–7% depending on the strain and conditions for cultivation. This is probably the cause of the rise in fat content.
It is essential to note that the standard deviations provided indicate some variability in the fat content measurements, Nonetheless, the overall trend of increased fat content with spirulina supplementation is clear from the data.

The Total Phenolic Content increases with the incorporation of spirulina in gluten-free pasta. There is a clear trend showing that higher spirulina concentrations lead to higher phenolic content, enhancing the pasta's antioxidant capacity. These findings indicate that spirulina supplementation not only improves gluten-free pasta's nutritional profile but also significantly boosts its phenolic content, potentially offering greater health benefits.

The statistical analysis indicates that supplementing rice and quinoa gluten-free pasta with 10% and 15% spirulina results in considerable increases in protein, ash, fat, and phenolic content, while 5% supplementation only shows significant increases in ash and fat. Moisture content is not significantly affected by any of the spirulina supplementation levels.

SEM results indicate that in control sample which had only 70% rice flour and 30% quinoa flour there was a dense, compact, continuous matrix, and showed good protein-starch interaction. But at 5% Spirulina addition of Spirulina there was an Introduction of fibrous structure and slight disruption in the matrix. At 10% Spirulina supplementation there was Increased porosity and more significant disruption of the protein-starch matrix seen. When 15% Spirulina was added to the70% rice and 15% quinoa flour there was highly porous, irregular structure seen with a severely compromised matrix.

It means that the addition of spirulina affects the structural integrity and morphology of the gluten-free pasta, with higher spirulina concentrations leading to more significant disruptions in the protein-starch matrix. When the percentage of spirulina increases the percentage of quinoa decreases in the dough which is serving as a binding agent to pasta starts losing its integrity. In gluten free pasta, because of the absence of gluten and other proteins, there are only albumins and globulins proteins from Quinoa, and Methionine and lysine-like essential amino acids from spirulina to make a dense and smooth pasta matrix as shown in micrograph Fig 1. Albumins and globulins constitute the primary protein component in quinoa, accounting for 44-77% of the overall protein content. Due to hydrogen Bonding the hydroxyl groups of starch interact with the amino and carboxyl groups of proteins, forming a mesh of hydrogen bonds that contribute to the structural integrity of the pasta. This suggests that 5-10 % of spirulina supplementation, has a good impact on the structural properties of gluten-free pasta for further addition needs careful consideration.

Gluten intolerance is a crucial health problem that necessitates meticulous management and carries substantial implications for people, the healthcare system, and society at large. The rice and quinoa flour are as an alternative ingredient to wheat-based pasta and the addition of spirulina to the pasta enhanced consciousness and contributed to enhancing the quality of life for individuals impacted by gluten intolerant disorder. So, it shall be a healthy addition in the gluten free market. The product can be exported but can be easily made in household as a better alternative to standard pasta products. It is advised that an in vitro protein and starch digestibility test be performed on the gluten-free noodles enhanced with spirulina algae, as one has not yet been done. Sensory analysis of the product may be a useful tool to vary the percentage of spirulina as per market segment identified.

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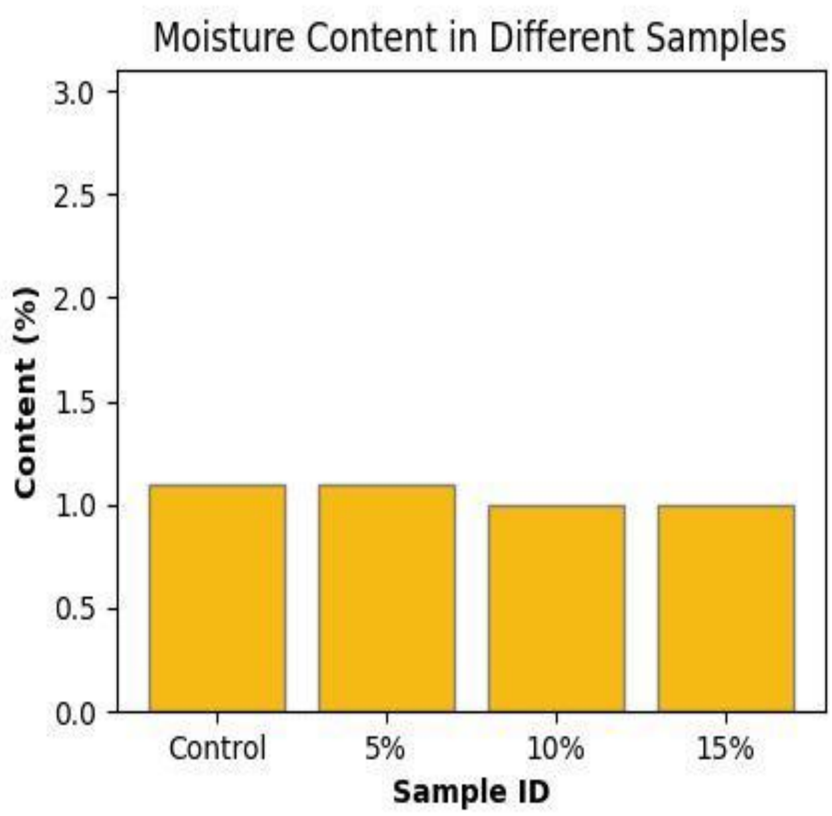
APPENDIX A

A-1 Moisture Content Test Readings

Column1	Column2	Column3	Column4	Column
	Sample wt +Crucible Wt (Wet)	Sample wt +Crucible Wt (Dry)	Results (MC%)	Mean
Sample (Control)	21.69	21.44	1.18	1.17
Sample (Control)	21.69	21.45	1.13	
Sample (Control)	21.76	21.5	1.22	
Sample (5%)	21.48	21.24	1.11	1.13
Sample (5%)	21.55	21.3	1.15	
Sample (5%)	21.25	21.01	1.12	
Sample (10%)	21.62	21.39	1.08	1.07
Sample (10%)	21.72	21.48	1.12	
Sample (10%)	21.57	21.35	1.03	
Sample (15%)	21.68	21.46	1.03	1.02
Sample (15%)	21.48	21.266	1.01	
Sample (15%)	21.72	21.5	1.02	

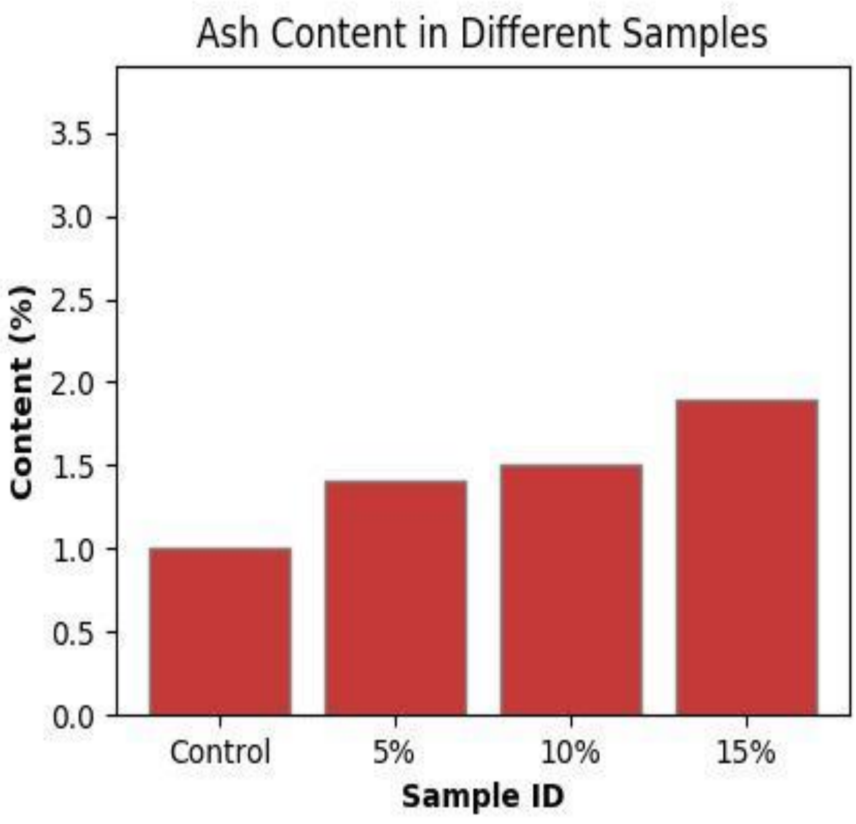


A-2 Ash Content Test Readings

% ASH = ((ashed wt.) - (crucible wt.)) x 100/((crucible and sample wt.) - (crucible wt.))

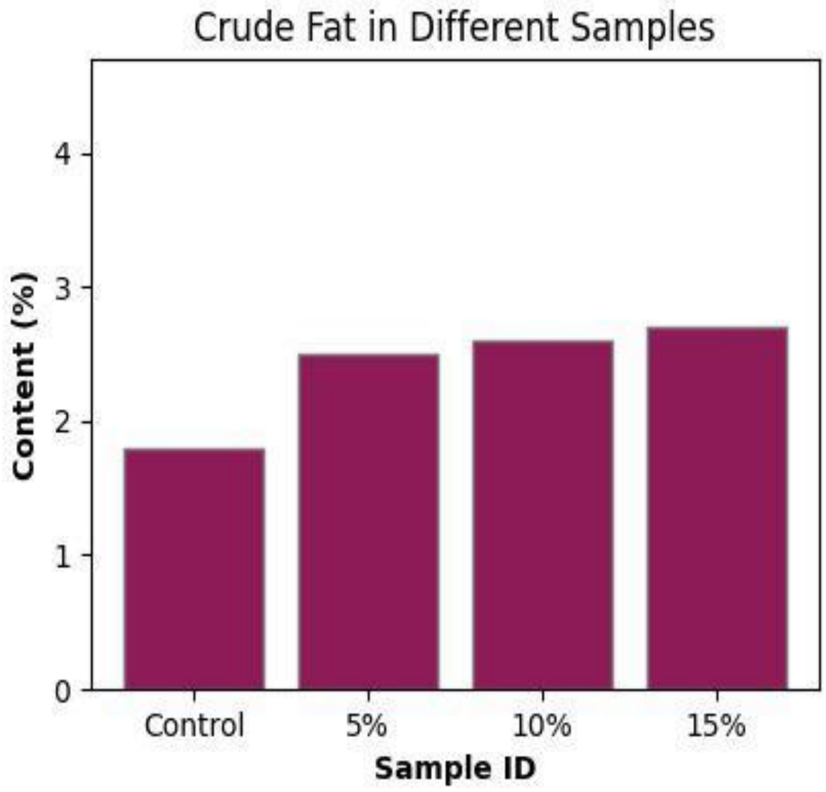
Temperature=500C
Time=3 hrs

Column1	Column2	Column3	Column4	Column5	Column6
	Ashed Wt	Crucible Wt	Sample wt	Crucible+Sample wt	**Results (Ash%)**
Sample (Control)	19.80	19.77	2.01	21.78	**1.49**
Sample (5%)	21.34	21.11	2.07	23.18	**0.99**
Sample (10%)	19.45	19.42	2.10	21.52	**1.43**
Sample (15%)	20.22	20.18	2.09	22.27	**1.91**



A-3 Crude Fat Test Readings

Replicates	Sample Name	Weight of sample (Ws)	Weight of Empty Flask (W1)	Weight of flask with Extract (W2)	Fat (%)
1	Sample (Control)	8.10	161.87	162.01	1.73
2	Sample (Control)	7.95	160.9	161.06	2.01
3	Sample (Control)	7.90	161.45	161.6	1.90
1	Sample (5%)	8.00	160.77	161	2.87
2	Sample (5%)	8.00	159.96	160.17	2.62
3	Sample (5%)	8.14	154.88	155.08	2.46
1	Sample (10%)	8.12	157.8	158.01	2.59
2	Sample (10%)	7.65	162.1	162.31	2.75
3	Sample (10%)	7.88	162.89	163.11	2.79
1	Sample (15%)	8.09	153.26	153.44	2.22
2	Sample (15%)	8.05	160.4	160.62	2.73
3	Sample (15%)	7.99	156.8	157.01	2.63

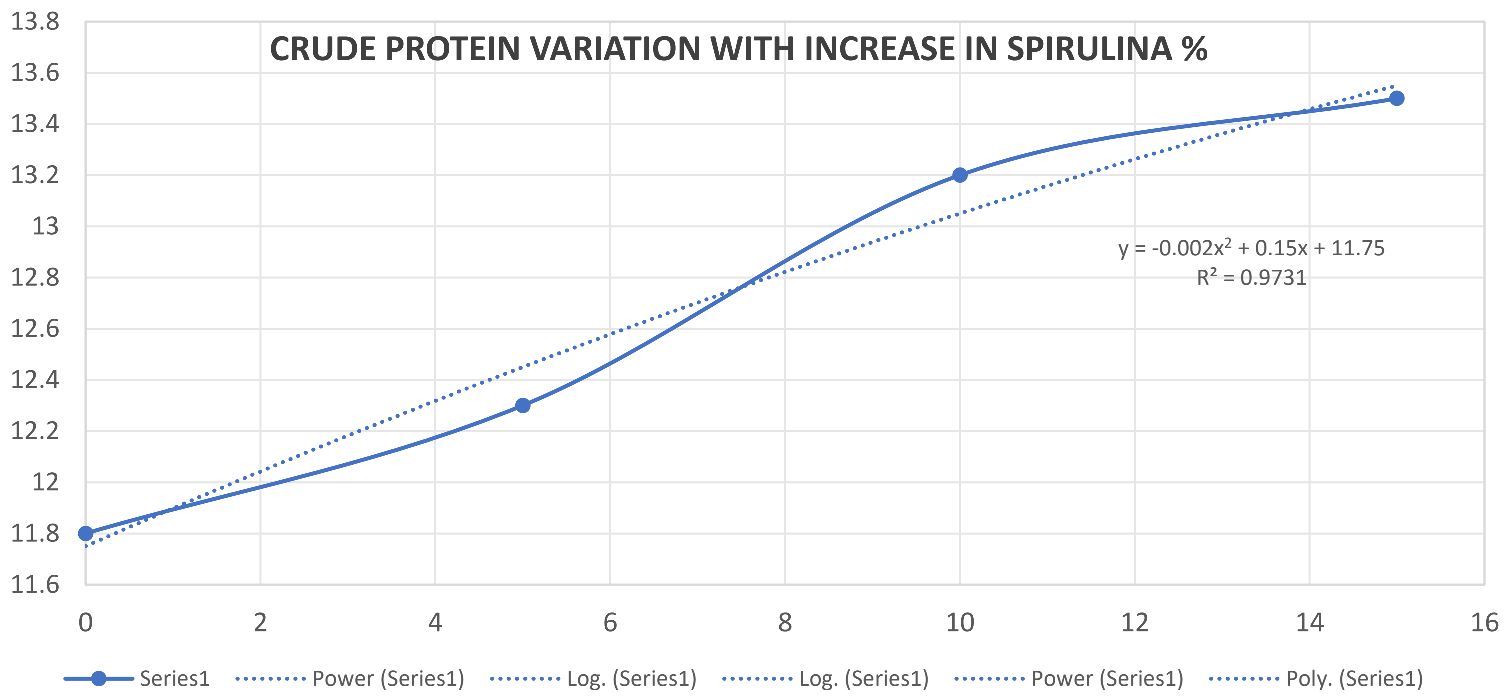


A-4 Crude Protein Test Readings:

4	15%	0.2705	0.1415	0.7075

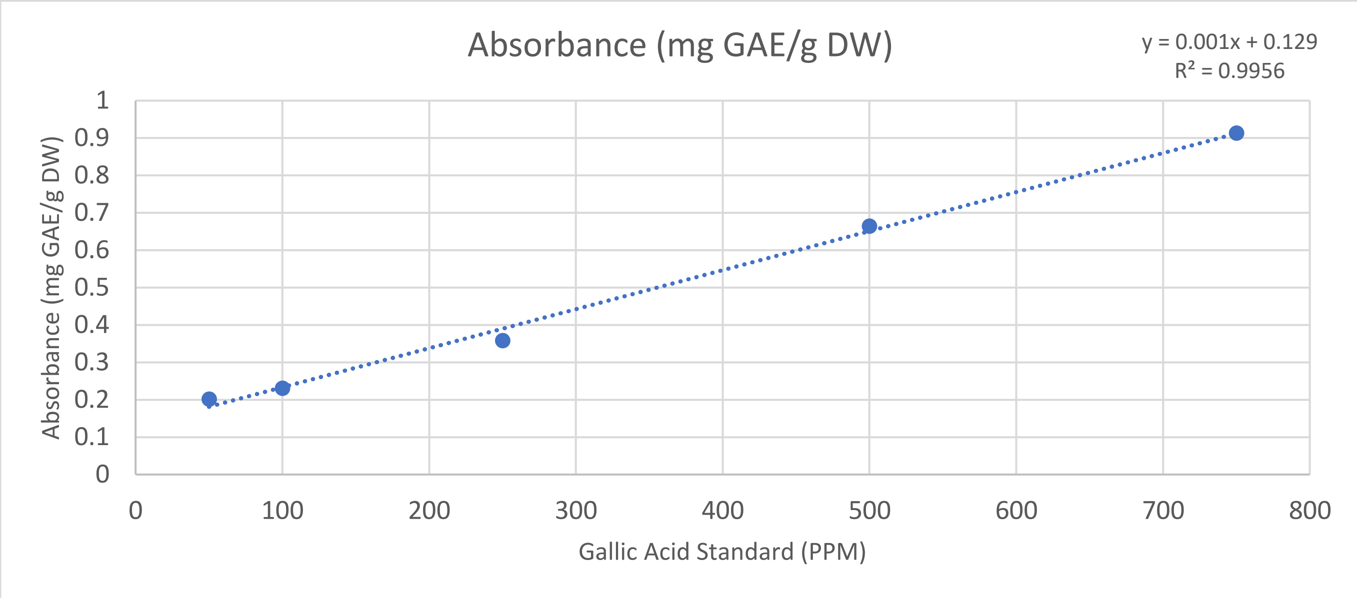
Crude Protein Content Result:

Sr. no	Replicates	Sample Name	Sample Quantity (g)	Nitrogen	Protein factor	Crude Protein (%)
1	1	Sample (Control)	1.011	2.14	6.25	**11.75**
2	2	Sample (Control)	1.003	2.10	6.25	**12.31**
3	3	Sample (Control)	1.000	2.11	6.25	**11.56**
4	1	Sample (5%)	1.001	1.99	6.25	**11.90**
5	2	Sample (5%)	1.000	1.98	6.25	**12.20**
6	3	Sample (5%)	1.022	2.03	6.25	**12.83**
7	1	Sample (10%)	1.006	1.88	6.25	**12.90**
8	2	Sample (10%)	1.014	1.97	6.25	**13.20**
9	3	Sample (10%)	1.000	1.85	6.25	**13.60**
10	1	Sample (15%)	1.002	1.87	6.25	**13.30**
11	2	Sample (15%)	1.012	2.05	6.25	**13.50**
12	3	Sample (15%)	1.001	1.82	6.25	**13.82**



A-5 Phenolic Content Readings

Total Phenolic Content Results:



	STANDARDS (PPM)	ABSORBANCE (MG GAE/G DW)
	50	0.2018
	100	0.2309
	250	0.3576
	500	0.6645
	750	0.9128

	Sample Name	Absorbance	Trend	Results TPC (mg GAE/g DW)
1	Control	0.1447	0.0157	0.0785
2	5%	0.1548	0.0258	0.129
3	10%	0.2424	0.1134	0.567