

**FORMULATION OF QUINOA MILLET BISCUIT INCORPORATED WITH
PREBOITIC FOR IRON DEFICIENCY PATIENT**

PROJECT REPORT

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science in
CLINICAL NUTRITION AND DIETETICS

By

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**DEPARTMENT OF CLINICAL NUTRITION AND DIETETICS
SCHOOL OF ALLIED HEALTH SCIENCES**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

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BONA FIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of E.RUTH KEZIAH (41736014) who have done the project work as a team E. RUTH KEZIAH (41736014) AND S.RAGAVI (41736013) who carried out the project entitled "FORMULATION OF QUINOA MILLET BISCUIT INCORPORATED WITH PREBOITIC FOR IRON DEFICIENCY PATIENT" under our supervision from July 2023 to April 2024.

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DECLARATION

I am E.RUTH KEZIAH(41736014) hereby declare that the Project Report entitled "FORMULATION OF QUINOA MILLET BISCUIT INCORPORATED WITH PREBOITIC FOR IRON DEFICIENCY PATIENT "done by us under the guidance of Dr. (Mrs) GAYATHRI. P at the Department of Clinical Nutrition & Dietetics, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition & Dietetics.

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DECLARATION

We S. RAGAVI (41736013) and E.RUTH KEZIAH (41736014) hereby declare that the Project Report entitled **"FORMULATION OF QUINOA MILLET BISCUIT INCORPORATED WITH PREBOITIC FOR IRON DEFICIENCY PATIENT"** done by us under the guidance of Dr. (Mrs) GAYATHRI P at the Department of Clinical Nutrition & Dietetics, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition & Dietetics.

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ABSTRACT

The most common form of anemia in the world is Iron-deficiency anemia. Anemia is defined as the clinical state where the blood lacks healthy red blood cells and this result in decreased oxygen flow to different organs of the body. Iron deficiency anemia is occurred due to insufficient iron. Without adequate iron, the body cannot produce enough of hemoglobin which is a vital part of red blood cells and thus it causes impaired oxygen supply.

The aim of investigation to produce high quality, nutritional value, sensory properties biscuits from quinoa flour QF, flaxseed FS and wheat flour WF. The materials used in this investigation were QF, FS and WF as well as other ingredients used to produce biscuits.

Cookies were subjected to analysis as Energy, protein, carbohydrates, fat in testing for macronutrients as well as mineral analysis (potassium, calcium, sodium and iron) and evaluated for consumer acceptance by sensory analysis.

The proximate analysis of the product revealed that it comprises of 495g/100g of energy, 65.9g/100g of carbohydrates, 21.5g/100g of total fat, 9.54g/100g of protein, 6.81g/100g of dietary fiber, iron 3.74g/100g with hedonic rating the maximum and minimum overall acceptability is 4.3 and 4.5 respectively.

During the study, it was observed that Quinoa millet biscuit had a IRON level of 3.74mg/100gm.

Keywords: quinoa flour, flaxseed, wheat flour.

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CHAPTER -1

INTRODUCTION

Anemia is characterized by a clinical state wherein the blood lacks sufficient healthy red blood cells, leading to decreased oxygen flow to various organs of the body. Iron deficiency anemia occurs due to insufficient iron levels. Inadequate iron hampers the production of hemoglobin, a crucial component of red blood cells, resulting in impaired oxygen supply. Consequently, individuals may experience fatigue and shortness of breath. Iron deficiency can disrupt biological functions such as respiration, energy production, DNA synthesis, and cell proliferation.

Iron-deficiency anemia is the most prevalent form of anemia globally. Iron deficiency and iron deficiency anemia are among the most prevalent medical conditions and global health challenges encountered in everyday clinical practice. Iron deficiency remains a leading cause of anemia worldwide, significantly impacting the lives of young children and premenopausal women in both developed and low-income countries. Enhancing the diagnosis and treatment of this condition is imperative. (Prolay Paul *et al.*, 2021).

Iron deficiency and anemia of chronic disorders commonly coexist and are observed in elderly patients and those with chronic kidney disease. (susan.f clark., 2018) In adults, iron-deficiency anemia can lead to a wide array of adverse outcomes, such as diminished work or exercise capacity, impaired thermoregulation, compromised immune function, gastrointestinal disturbances, and neurocognitive impairment.

Globally, approximately 1.62 billion people are affected by anemia, accounting for around 24.8% of the population. The highest prevalence, at 47.4%, is observed among preschool-aged children, while the lowest occurrence is found in men, at 12.7%. Among all population groups, non-pregnant women are the most affected, with an estimated 468.4 million individuals affected.

One gram of iron is stored in the liver, with 0.4 grams in myoglobin and cytochromes. A small amount, approximately 3 mg, circulates bound to plasma

transferrin. Men and non-menstruating women typically lose around 1 mg of body iron per day, while menstruating women may lose an additional 1 mg daily on average. Full-term babies are born with 180 mg of iron, but they need to double their red cell mass within 12 months (low birth weight infants need to more than double their red cell mass). Iron requirements increase significantly during adolescence due to the rise in blood volume and lean body mass, which is further compounded in females by the onset of menstruation and during pregnancy by increases in maternal red cell mass and fetal erythropoietin.

Dietary iron consists of haem iron from animal sources and non-haem iron from cereals and vegetables. Haem iron, bound to hemoglobin and myoglobin, is more efficiently absorbed than non-haem iron. Non-haem iron is absorbed by intestinal luminal cells through a specific transporter, the divalent metal transporter located on the apical membrane of intestinal enterocytes. It is then released into circulation, where it binds to transferrin. Erythroblasts express transferrin receptors that accept iron-transferrin complexes, undergo endocytosis, and incorporate iron into hemoglobin. While the precise mechanism of haem-iron absorption remains unclear, potential transporters have been identified. (West AR, 2008;)

Enhancing dietary diversity to boost micronutrient intake represents the most favorable and sustainable strategy for preventing micronutrient malnutrition, (DeMaeyer EM, 1989) despite the superior absorption of heme iron from animal sources compared to non-heme iron from plant sources. Whole cereals, pulses, and vegetables, especially green leafy vegetables (GLVs), play a significant role in providing dietary iron in Indian diets. (Park K, 2019)

Cookies constitute the largest segment of snack items in bakery products, appealing to individuals across all economic backgrounds and age groups due to their affordability, extended shelf life, ease of consumption, and nutritional value. Baked for easy digestion and convenient portability, cookies are favored as on-the-go snacks during travel. According to statistical data, cookies rank as the second most consumed baked food product at 17.3%, following bread. Widely embraced worldwide, cookies offer a rich source of nutritional components and are produced in various types, including sugar-free, protein-

enriched, wheat germ and multigrain varieties. While refined flour, sugar, and fat are the primary ingredients in cookie production, minor components like flavonoids and additives are also utilized. Although synthetic colorants pose no health risks, there's a growing trend in the food industry towards natural colors in response to consumer demand. Natural colors offer numerous health benefits, including a diverse array of vitamins, minerals, nutritional value, and antioxidant properties. (Sunita Mishra, 2020)

Millets, among the oldest foods known to humanity, are likely the earliest cereal grains cultivated for domestic use. These small-seeded grasses exhibit remarkable hardiness and thrive in arid regions as rain-fed crops, even in conditions of low soil fertility and moisture. Their unique attributes distinguish millets from other cereals, as they can withstand dry climates, thrive in poor soils, and require minimal external inputs for growth. With superior nutritional profiles compared to conventional cereals, millets serve as staple foods in many households. India stands as the world's largest producer of millets, underscoring their importance in global agriculture. Properly stored, whole millets can maintain their quality for two years or longer. Baked goods predominantly rely on cereal flours blended with various ingredients. However, due to its lack of gluten, millet protein is unsuitable as the sole component for baking products.

The term "multigrain" flour denotes that the bread comprises various types of grains, setting it apart from whole grain breads. These grains may encompass oats, barley, cornmeal, wheat, millet, flax, and others. Nevertheless, there isn't a definitive list of grains required for a product labeled as "multigrain." Moreover, only a minimum of two different grains is necessary for a product to qualify as "multigrain" flour. Furthermore, the term doesn't specify how the grains should be processed. Hence, "multigrain" doesn't always equate to "whole grain." (Dhirendra Kumar Dinkar *et al*, July-2020)

Quinoa, scientifically known as *Chenopodium quinoa*, is classified as both a pseudo-cereal and pseudo-oil seeds crop, belonging to the family "Chenopodiaceae". Cultivated for many years in Andean regions, it is a dicot

plant reaching heights of 1-3m from the soil. Quinoa thrives in various soil types due to its extensive root system, extending up to 30cm. Remarkably, it can produce grains even when sown at altitudes of up to 4500 meters, boasting superior nutritional value compared to other cereal crops. Typically, quinoa exhibits cylindrical stems with a diameter of approximately 3.5cm, with variations in color among different varieties. Its leaves resemble those of a goose foot. The seeds of quinoa are round and flat, with a diameter ranging from 1.5 to 4.0 mm, and weigh about 1 g per 350 seeds. (Daraz. O *et al*, 2020)

The color of quinoa seeds varies from white to grey and black. Worldwide, there are nearly 250 varieties of quinoa, classified based on plant morphology, phenology, and physical appearance, with some consideration given to tissue chemical composition. (Mujica A., 1994)

Flax (*Linum usitatissimum*; Linaceae), commonly known as linseed, is primarily cultivated for its seeds and fiber. Its dark brown seeds are utilized in the food industry, often added to cookies bread and cakes. With an omega-3 fatty acid content of around 60%, flaxseed stands as one of the richest vegetarian sources of this compound, also containing omega-6 fatty acids. Incorporating flaxseed into one's diet provides appropriate levels of polyunsaturated fatty acids and plays a significant role in preventing and regulating cardiac and autoimmune diseases, as well as breast, prostate, and colon cancer. (Cunnane *et al*, 1993)

Cookies constitute the largest segment of snack items in bakery products, appealing to individuals across all economic backgrounds and age groups due to their affordability, extended shelf life, ease of consumption, and nutritional value. Baked for easy digestion and convenient portability, cookies are favored as on-the-go snacks during travel. According to statistical data, cookies rank as the second most consumed baked food product at 17.3%, following bread. Widely embraced worldwide, cookies offer a rich source of nutritional components and are produced in various types, including sugar-free, protein-enriched, wheat germ and multigrain varieties. While refined flour, sugar, and fat are the primary ingredients in cookie production, minor components like flavonoids and additives are also utilized. Although synthetic colorants pose no

health risks, there's a growing trend in the food industry towards natural colors in response to consumer demand. Natural colors offer numerous health benefits, including a diverse array of vitamins, minerals, nutritional value, and antioxidant properties. (Sunita Mishra, 2020)

Quinoa grains possess beneficial health attributes and can be utilized as a remedy for various diseases across all consumer groups. Individuals may suffer from conditions such as osteoporosis in women, anemia, obesity, and celiac disease. (Zevallos *et al.*, 2015)

Quinoa seeds have a long history of consumption, particularly in South America, where they are traditionally utilized. Similar to other cereals like rice, quinoa seeds are commonly used in soup preparations. Additionally, they serve as a breakfast cereal when puffed and are also processed into flour for various bakery products, including cookies, biscuits, breads and cakes. (Bhargava, 2006)

Functional foods encompass any substance or component within food that provides health benefits, aiding in the prevention and treatment of diseases. Key functional components of foods include fibers, polyunsaturated fatty acids, phytochemicals, active peptides, prebiotics, and probiotics. (Kucuk, 2002) While the human body synthesizes most saturated and unsaturated fatty acids, it does not produce essential ones such as omega-3 (linolenic acid) and omega-6 (linolenic acid). (Soccol *et al*, 2003)

Baking, a culinary method employing dry heat typically in an oven, or alternatively, in hot ashes or on hot stones, is primarily known for its application in producing bread but extends to various other food items. The gradual transfer of heat from the exterior to the interior transforms batters and doughs into finished goods with a crisp outer layer and a moist center. This technique can be combined with grilling to create a hybrid variation, utilizing both methods simultaneously or sequentially. Baking shares similarities with grilling, as the concept of the oven parallels that of a smoke pit. (Smita Kagwad *et al*, June 2019)

A functional food is characterized as a modified food or food ingredient that offers potential health benefits beyond its basic nutrients. Bakery products can be enriched with health-promoting active ingredients to create non-dairy-based functional foods. Ensuring the viability of these active components, such as probiotics, in the final products is essential to imparting beneficial effects on consumer health. However, the baking process presents a hurdle in maintaining the functionality of these heat-sensitive active ingredients due to the elevated temperatures involved. (Zhang, 2018)

The primary objective of defining marketing and tracing its evolution is to pinpoint the foundational field from which the study of consumer behavior emerged as a critical area of focus. Marketing is delineated as the process of identifying, foreseeing, and analyzing consumer needs and desires, with marketers striving to comprehend these needs and offer solutions in the form of goods or services. This definition underscores the central roles of marketers, organizations, and customers within the business landscape. In 1935, the American Marketing Association (AMA) provided a succinct definition of marketing as the systematic performance of business activities aimed at managing customer relationships, which emphasizes a comprehensive understanding of both business operations and customer dynamics.

Subsequently, in 1985, the AMA expanded this definition to include marketing's involvement in pricing, promoting, and distributing products to fulfill customer needs and desires, thus elucidating the fundamental principles of marketing theory and execution. It is elucidated that marketing operates at both managerial and societal levels, entailing the scientific selection of markets, the persuasion and retention of customer bases, and the facilitation of societal and consumer participation in value creation, provision, and exchange. Across these definitions, the pivotal role of the customer is highlighted, emphasizing the imperative nature of understanding consumer behavior for effective strategy formulation. (Maxwell Chufama, 2021,)

CHAPTER-2

REVIEW OF LITERATURE

Prolay Paul *et al.*, have reported that Anemia is characterized by a clinical state in which the blood lacks sufficient healthy red blood cells, leading to decreased oxygen delivery to various organs in the body. Iron deficiency anemia arises from insufficient iron levels, hindering the body's ability to produce an adequate amount of hemoglobin, a crucial component of red blood cells necessary for oxygen transport. This condition is more prevalent in women, particularly during menstruation and pregnancy, due to increased iron demands. Various factors, including physiological issues such as poor digestive function, contribute to iron deficiency. Insufficient iron leads to a deficiency in hemoglobin impairing oxygen delivery, as the bone marrow struggles to produce an ample supply.

Most cases of iron deficiency anemia are mild and easily treatable, untreated cases can lead to complications. Iron supplementation, typically taken on an empty stomach for optimal absorption, helps replenish iron levels in the body. However, if adverse effects occur, supplements can be taken after meals. Treatment often requires continued supplementation for several months, although these supplements may cause side effects such as constipation or black stools. Drug-induced iron deficiency anemia is a blood disorder triggered by medications that prompt the immune system to attack red blood cells prematurely, leading to their breakdown, a process known as hemolysis (Prolay Paul *et al.*, 08 august 2021)

Shashi Bhushan Kumar *et al.*, have reported that Iron deficiency anemia has reached epidemic proportions in developing countries and has become a major global public health concern, particularly affecting 0-5-year-old children and young women of childbearing age, especially during pregnancy. Iron deficiency can lead to life-threatening depletion of red blood cells, muscle function, and energy production, thereby causing weakness and impairing growth, motor skills, and cognitive performance. Iron deficiency anemia significantly impacts the well-being of the younger population and impedes the economic progress of developing nations like India. The etiology of Iron deficiency anemia involves an imbalance between iron intake/absorption/storage and iron utilization/loss.

Shashi Bhushan Kumar *et al.*, have reported that Despite numerous strategic programs aimed at increasing iron intake, simply improving iron intake alone has proven insufficient to effectively combat Iron deficiency anemia. Emerging critical risk factors for Iron deficiency anemia encompass various elements such as cultural dietary habits, infections, genetics, inflammatory conditions, metabolic disorders, dysbiosis, and socioeconomic factors. This review explores various Iron deficiency anemia mitigation initiatives in India and their associated limitations. The multifactorial nature of Iron deficiency anemia pathogenesis offers new avenues for enhancing mitigation programs and alleviating Iron deficiency anemia in India and globally (Shashi Bhushan Kumar *et al.*, 20 July 2022).

Shekhara Naik R *et al.*, reported that The role of flaxseed as a functional food continues to gain momentum, particularly in the cardiovascular health, owing to its rich content of alpha-linolenic acid, lignans, and soluble fiber. Flaxseed contains secoisolariciresinol diglucoside, a lignin derivative, which metabolizes into mammalian lignans, influencing early risk markers of mammary and colonic carcinogenesis in animal models.

Regular consumption of flaxseed products can impact serum cholesterol levels, glucose absorption, inflammation markers, and omega-3 fatty acid levels. Flaxseed exhibits significant antioxidant and anti-inflammatory properties in both experimental and human studies, with supplementation showing potential benefits in cardiovascular risk, certain cancers, and metabolic disorders. Numerous studies underscore the health-promoting and disease-preventing potential of raw flaxseed and its baked products, positioning it as a promising nutraceutical with protective and therapeutic properties (Shekhara Naik R *et al.*, 08 July 2020)

Charu Katare *et al.*, have reported that The surge in recognition of flax as a functional food continues, with recent attention focused on its potential benefits for cardiovascular health due to its rich content of alpha-linolenic acid, lignans, and soluble fiber. The potential of flax seed as a nutraceutical and its role as a protective and therapeutic medicinal food are underscored in this review (Charu Katare *et al.*, 2012)

Nihir Soni *et al.*, have reported that The study conducted aimed to determine

the most suitable blend of pulses flour for partially replacing refined wheat flour in cookies to enhance their protein content. Soybean, Moth bean, and Chickpea were identified as potential protein sources with sufficient biological value based on existing literature. Different replacement levels (10, 15, 20, and 25 per cent) of these flours were tested in the study. Cookies were then developed using these blends and subjected to analysis including physical, chemical, textural, and sensory evaluations. Following the analysis, cookies containing 20% Chickpea and 80% refined wheat flour were selected, resulting in an increase in protein content from 2.9% to 6.8% (Nihir Soni *et al.*, 2018)

PP Salunke *et al.*, have reported that The present research aimed to investigate the potential use of barnyard millet, an underutilized yet highly nutrient-rich grain, in cookies. Initial experiments were conducted to determine the optimal blend of barnyard millet flour with maida and wheat flour for producing high-quality cookies. Three formulations were selected: 50% maida and 50% wheat flour, 30% maida and 70% barnyard millet, and 20% wheat flour and 80% barnyard millet flour. These formulations were then packed in LDPE and stored at ambient temperature for 90 days to assess their storage stability.

The chemical composition of the fresh cookies varied across formulations. For MWF50, the moisture content was 4.0%, protein content was 11.55%, crude fat was 26.30%, crude fiber was 1.10%, carbohydrates were 71.65%, calcium was 35.50 mg/100 g, phosphorus was 238 mg/100g, and iron was 3.80 mg/100 g. For MBF70, the moisture content was 3.10%, protein content was 7.64%, crude fat was 26.81%, crude fiber was 6.95%, carbohydrates were 68.02%, calcium was 20.90 mg/100 g, phosphorus was 232 mg/100g, and iron was 4.31 mg/100 g. For WBF80, the moisture content was 3.0%, protein content was 7.38%, crude fat was 27.10%, crude fiber was 8.22%, carbohydrates were 66.28%, calcium was 30.60 mg/100 g, phosphorus was 295 mg/100g, and iron was 4.98 mg/100 g.

Sensory evaluation of the cookies was conducted monthly for three months during storage. Overall acceptability scores were affected by storage duration. The results showed a decrease in overall acceptability scores for the control (from 7.35 to 7.10), MWF50 (from 7.50 to 7.15), MBF70 (from 8.45 to 8.00), and WBF80 (from 8.63 to 8.15) formulations in LDPE over 90 days of storage. However, the storage study

demonstrated that cookies incorporating maida, wheat, and barnyard millet flour could be stored for up to three months in LDPE with minimal losses in sensory, nutritional, and textural characteristics. There were no significant differences in protein, crude fiber, calcium, and iron content with the advancement of the storage period over three months. The cookies remained acceptable for consumption even after three months of storage at ambient temperature (PP Salunke *et al.*, 30 June 2019)

Viktoria Angeli *et al.*, have reported that Quinoa (*Chenopodium quinoa* Willd.) is indigenous to the Andean region and has garnered global interest due to its exceptional nutritional value, boasting higher protein content compared to other cereals and a superior distribution of essential amino acids. Its versatility extends to serving as an alternative to milk proteins. Moreover, quinoa is rich in essential fatty acids, minerals, vitamins, dietary fibers, and carbohydrates with advantageous hypoglycemic effects, all while being gluten-free. The resilience of the quinoa plant against cold, salt, and drought has earned it the moniker "golden grain," driving its increasing production and product diversification, particularly highlighted during its designation as the international year of quinoa in 2013. This review comprehensively explores the nutritional and biological attributes of quinoa cultivated worldwide over the past two decades, delving into the evolution of traditional processing methods and products, their integration into modern food processing, and the potential utilization of quinoa by-products across various industrial sectors. Additionally, it addresses socio-economic and environmental challenges associated with quinoa's production, consumption, and marketization, advocating for a holistic approach to promoting quinoa's cultivation and consumption (Viktoria Angeli *et al.*, 19 February 2020)

Mariane Lutz *et al.*, reported that The resurgence of interest in quinoa and its reintroduction into the diet is motivated by the current epidemiological landscape, characterized by diseases with risk factors that can be mitigated through a balanced and nutritious diet, with quinoa being a prominent component due to its status as a "superfood." Additionally, it has been designated as one of the crops prioritized by the Food and Agriculture Organization (FAO) to enhance food security (Mariane Lutz *et al.*, 2017)

Vandana et al., reported that The raw and processed quinoa grains has been examined. Biscuits were formulated using varying ratios of refined wheat flour and quinoa flour, namely 100:0, 75:25, 50:50, 25:75, and 0:100. These biscuits were then evaluated for their physical, textural, sensory, and nutritional qualities. The physical characteristics of the biscuits indicated an increase in product thickness, weight, and density with the incorporation of quinoa flour, while diameter, spread ratio, and volume decreased. Changes in textural characteristics were also noted, with hardness values increasing as the proportion of quinoa flour in the recipe increased. Sensory evaluation results revealed that biscuits containing up to 75 percent quinoa flour were acceptable to panelists. Furthermore, the nutrient composition of the biscuits demonstrated that protein, fat, fiber, and energy content increased as the level of quinoa (Vandana *et al.*, 2020)

Dorna Davani-Davari *et al.*, reported that Prebiotics, a category of nutrients broken down by gut microbiota, have garnered increasing interest due to their potential impact on overall human health. By nourishing intestinal microbiota, they generate short-chain fatty acids that enter the bloodstream, influencing not just gastrointestinal health but also distant organs. Fructo-oligosaccharides and galacto-oligosaccharides stand out as pivotal prebiotic groups with significant health benefits. Given their natural occurrence in low quantities in foods, scientists are exploring methods to produce prebiotics on an industrial scale. With their demonstrated safety and advantages in production and storage over probiotics, prebiotics emerge as compelling candidates for enhancing human health either as substitutes for or in conjunction with probiotics. This review explores various facets of prebiotics, shedding light on their indispensable role in human well-being (Dorna Davani-Davari *et al.*, 09 march 2019)

Thaisa Menezes Alves Moro *et al.*, reported that Burdock roots, rich in fructooligosaccharides and phenolic compounds, are underutilized as a food ingredient. This study aimed to produce burdock root flour (BRF) through conventional drying and assess its viability as a prebiotic ingredient in cookies. The cookies were made by substituting refined wheat flour with BRF at 0% (C0), 5% (C1), and 10% (C2) concentrations in a blend with whole wheat flour. BRF contained $40.16 \text{ g} \times 100 \text{ g}^{-1}$ of total dietary fiber, $181.74 \text{ mg} \times 100 \text{ g}^{-1}$ of chlorogenic acid, and $6.19 \text{ g} \times 100 \text{ g}^{-1}$ of total fructooligosaccharides (FOS), demonstrating in vitro

prebiotic activity. The cookies C1 and C2 contained 2.2% and 4.94% (on a dry basis) of BRF, respectively, and exhibited similar ($p > .05$) physicochemical and technological properties, except for the hardness of C1 (24.83 N), which was higher ($p < .05$) than C0 and C2. Incorporating BRF significantly increased ($p < .05$) FOS content in cookies from 42.38 (C0) to 366.68 mg $\times$ 100 g⁻¹ (C2). Cookies with 4.94% BRF showed a significant positive effect ($p < .05$) on prebiotic activity. Burdock roots present a promising alternative to introduce prebiotic oligosaccharides into bakery products, catering to consumers seeking functional foods with health benefits (Thaïsa Menezes Alves Moro *et al.*, 2018)

Sargun Kaur *et al.*, reported that The aim of this chapter is to deepen our understanding of consumer behavior and the factors shaping purchasing decisions, while also shedding light on diverse schools of thought that have influenced the evolution of consumer research. By exploring various dimensions found in the literature, we can effectively characterize and distinguish between different viewpoints on consumer research. This sets the stage for driving transformative shifts in future consumer research paradigms (Sargun Kaur *et al.*, 146-153)

Rosna Tadju *et al.*, reported that The study aimed to assess the sensory attributes of biscuit products fortified with a blend of sorghum flour and corn bran flour. Analyzing the data involved employing Analysis of Variance (ANOVA) to compare three treatment combinations: P1 (62% sorghum flour + 38% corn bran flour), P2 (72% sorghum flour + 28% corn bran flour), and P3 (83% sorghum flour + 17% corn bran flour). The sensory characteristics evaluated by the panelists encompassed texture, color, aroma, and taste. The findings revealed that the P3 treatment, featuring 83% sorghum flour and 17% corn bran flour, emerged as the preferred choice across all sensory parameters, including texture, color, aroma, and taste (Rosna Tadju *et al.*, 2021)

Khaled M *et al.*, reported that This work aimed at determining the maximum acceptable level of wheat germ that can be added to one of homemade biscuits in Jordan, studying some chemical and sensory properties of this product, moreover, studying the effect of storage time after two months at room temperature on the sensory parameters of this product. The results showed that the addition of wheat germ in biscuit making at level of 20% (w/w) enhanced the nutritional value by

increasing protein and total mineral content, as well as decreasing fat, total carbohydrates and total calories. The acceptability of sensory characteristics was improved by this fortification immediately after preparation and during storage (*Khaled M et al., 2017*)

Gamal Saad Elhadidy et al., reported that The investigation aimed to create high-quality biscuits with enhanced nutritional value, physical attributes, and sensory properties by incorporating quinoa flour (QF), naked barley flour (NBF), carrot powder (CP), and wheat flour (WF). The materials utilized included QF, NBF, CP, WF, and additional ingredients for biscuit production. Results revealed that formulations containing QF, NBF, and CP exhibited higher levels of ether extract, ash, and crude fiber compared to those using WF alone. Additionally, minerals such as potassium (K), calcium (Ca), phosphorus (P), and iron (Fe) were found in higher concentrations in NBF, QF, and CP compared to WF. Blends of naked barley, quinoa, and CP were also identified as rich sources of essential amino acids. Furthermore, biscuits formulated with NBF, QF, and CP (B2, B3, B4, and B5) demonstrated higher biological value (BV) or computed protein efficiency ratio (C-PER) compared to the control (WF). Through a carefully implemented physical process involving a well-blended combination of supplements, the study successfully produced biscuits with outstanding sensory properties, including taste, colour, aroma, and overall acceptability (*Gamal Saad Elhadidy et al., july 2020*)

CHAPTER-3

AIM AND OBJECTIVE

3.1 AIM

To prepare a quinoa millet biscuit incorporated with prebiotics for iron deficiency patient

3.2 OBJECTIVE

- To prepare the quinoa millet biscuit.
- To process and evaluate the nutritional analysis of the biscuit.
- To assess Microbiological testing of the biscuit (total plate count, yeast and mould, *E.coli*, Coliforms & *Salmonella*)
- Sensory evaluation of biscuit(view, smell, taste, touch & hear)
- Packing and Labelling

CHAPTER-4

MATERIALS AND METHODS

MATERIAL

4.1.1 COLLECTION OF RAW MATERIAL:

Flax seed and Quinoa millet was purchased from a local market in Chennai city. The Bakery ingredients such as wheat flour, brown sugar, butter, baking soda, baking powder, vanilla essence and cardamom, salt were procured from local grocery shop in Chennai city.

4.1.2 PREPARATION OF COOKIES:

The biscuits were prepared after the flour preparation, followed by standard formulation, with the addition of three different levels of wheat flour, and quinoa flour. Dry ingredients like (flax seed flour, quinoa flour, wheat flour, baking powder) were mixed and sieved twice for uniform mixing of leavening agents to the flour. A weighted amount of salt was added in the flour mixture.

A weighted amount of unsalted butter was taken in a bowl and beaten until it melts. Sugar was added and beat continuously for creaming. Flour mix was added in smaller amounts into the cream and uniformly mixed. The dough was rolled and then biscuits were cut into small round shape using a biscuit cutter. Biscuits were kept in an electric oven for 15 to 20 minutes at 180°C for uniform baking.

4.1.3 PREPARATION OF QUINOA FLOUR:

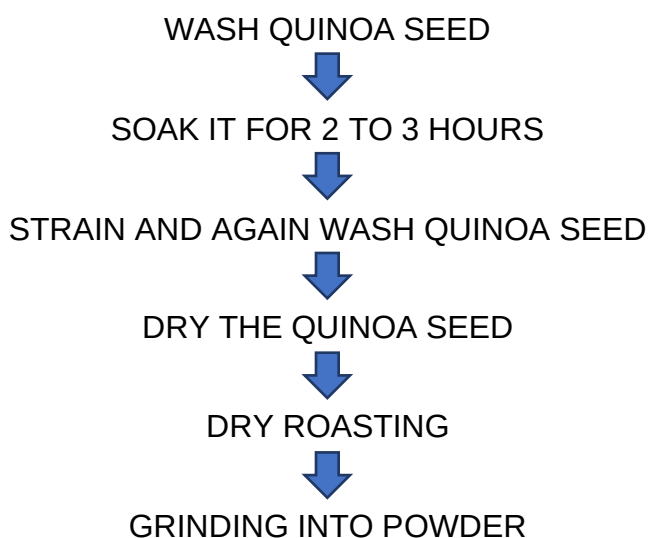


FIG 4.1: Flow diagram for preparation of Quinoa flour

4.1.4 PREPARATION OF QUINOA MILLET BISCUIT:

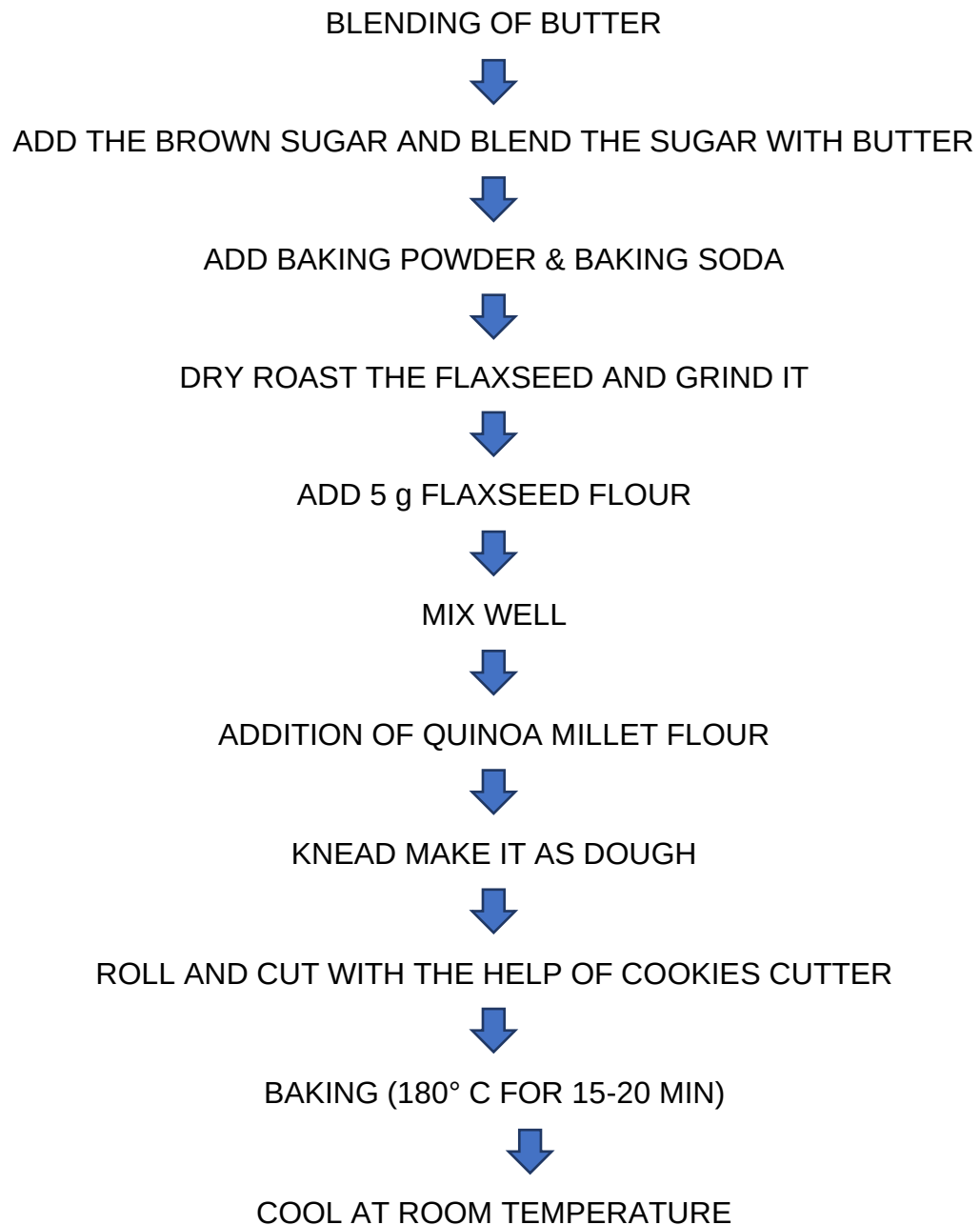


FIG4. 2: Flow diagram for preparation of Quinoa flour cookies

4.1.4 PREPARATION OF QUINOA MILET BISCUIT



Fig 4.3 DRYING

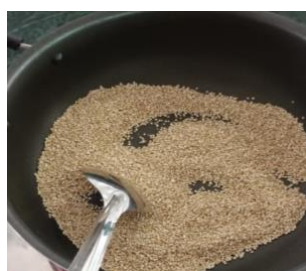


Fig 4.4 DRY ROASTING



Fig 4.5 GRINDING



Fig 4.6 ADDING
BAKING POWDER &
BAKING SODA



Fig 4.7 ADDING BROWN
SUGAR



Fig 4.8 BLENDING
BUTTER

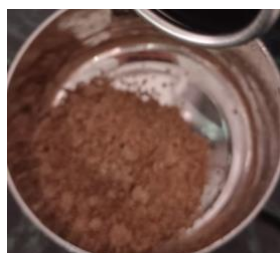


Fig 4.9 ADDING
FLAXSEED FLOUR



Fig 4.10 KNEAD



Fig 4.11 ROLL AND CUT



Fig 4.12 QUINOA MILLET BISCUIT

4.1.5 STANDARD FORMULATION OF COOKIES:

INGREDIENT	WEIGHT IN GM
Quinoa flour	120
Flax seed	5
Wheat flour	30
Butter	50
Brown sugar	50
Baking powder	0.1
Baking soda	0.1
Vanilla essence	5 Drops
Salt	0.1
Cardamom	1

4.2 METHODOLOGY

4.2.1 NUTRITIONAL EVALUATION ANALYSIS:

ENERGY

The caloric value was calculated in kilo-calories per 100g (kcal/100g) by multiplying the crude fat, protein and carbohydrate values by At water factors of 37, 17 and 17 respectively. This method is represented by the following formula.

$$\text{Energy (KCAL/100g)} = (\text{Carbohydrate} \times 4) + (\text{protein} \times 4) + (\text{fat} \times 9)$$

TOTAL CARBOHYDRATES

The total carbohydrate content in the quinoa millet biscuit was calculated by difference method. This methodology separately assesses the quantities of various other constituents present in the food, including protein, fat, fiber, moisture, and ash. These values are then aggregated and subtracted from the overall weight of the food. This method is represented by the following formula:

$$\text{Carbohydrate (g/100g)} = 100 - [\text{Moisture (\%)} + \text{Ash (\%)} + \text{Total fat (g)} + \text{Protein(g)} + \text{Fiber(g)}]$$

FAT

Use method A or C for feed ingredients and mixed feeds other than baked or expended, dried milk products, containing urea or mixed feeds that have at least 20% of crude fat derived from baked and expended or dried milk products.

- A. **Indirect methods:** determine moisture as in 934.01 or 920.36 then extract dried substance as in C And dried again. Report loss in weight as ether extract.

Direct methods:

- B. **Reagents:** Anhydrous ether: wash commercial ether with two or three portions H_2O , and solid NaOH or KOH, and let stand until most of H_2O is abstracted from the ether. Decant into Dry bottle, add small pieces of carefully cleaned metallic Na, and let stand until H_2 evolution ceases. Keep ether, thus dehydrated, over metallic Na in lossely stoppered bottles.
- C. **Determination:**(Large amounts H_2O – soluble components such as carbohydrates, urea, latucacid, glycerol and others may interfere with extraction of fat if present, extract 2 g test portions on small paper in funnel with five 20 mL portion H_2O prior to drying for ether extraction).

Extract ca 2 g test portions dried as in 934 with anhydrous ether. Extraction period may vary from 4 h at condensation rate of 5 – 6 drops /s to 16 h at 2-3 drops/ s. Dry extract 30 min at 100°C, cool and weigh.

PROTEIN

Weigh suitable test portion of sample into digestion flask. Add 10g of potassium sulphate or anhydrous sodium sulphate, about 0.4g $CuSO_4$ and 25mL or more, if necessary, of Conc. H_2SO_4 to the flask. Heat the flask for 90min for complete digestion. Cool, cautiously add 250mL H_2O and cool to room temperature. Add 100ml of 4% boric acid to the beaker, condenser tip will be sufficiently immersed. Add 3-4 drops of mixed indicator. Connect flask to distillation apparatus, add sufficient 45% NaOH solution, mix completely, such that mixture will be strongly alkaline and distil until 150ml distillate is collected in titration beaker. Titrate against 0.1N HCl solution. Correct for blank determination on reagents.

Calculation:

$$\%N (w/w) = (TVs-TVb) \times \text{Normality of standard HCl} \times 1.4007 / \text{Sample weight}$$

where, TVb = Titer value of blank; TVs = Titer value of sample

FIBER**Principle**

Duplicate test portion of dried foods, fat extracted if containing > 10% fat, are gelatinized with termally (heat stable α - amylase), and then enzymatically digested with protease amyloglucosidase to remove protein and starch. (When analyzing mixed diets, always extract fat prior to determining total dietary fiber.) Four volumes of ethyl alcohol are added to precipitate soluble dietary fiber. Total residue is filtered, washed with 78% ethyl alcohol, 95% ethyl alcohol, and acetone. After drying residue is weighed. One duplicate is analyzed for protein, and other is Incinerated at 525°C and ash is determined.

CALCULATION

Total dietary fiber = weight residue -weight (protein + ash)

Apparatus

- a) Fritted crucible- Porosity No. 2 (Pyrex No. 32940, coarse, ASTM 40-60 μ m; or Corning number and soak 529°C 36060 Buchner, fritted disk, Pyrex 60ML ASTM 40-60 μ m) Clean thoroughly heat 1 h at 525 °C and soak and then rinse in H₂O. Add ca 0.5 g Celite to air-dried crucibles and dry at v130°C to constant weight ($\geq$ 1h). Cool and store in desiccator until used.
- b) Vacuum source- Vacuum pump or aspirator equipped with in-line double vacuum flask to prevent contamination in case of H₂O
- c) Vacuum oven-70°C. Alternatively, 105°C air oven can be used.
- d) Desiccator
- e) Muffle furnace
- (f) Water baths – (1) Boiling (2) Constant temperature- Adjustable to 60°C, with either multi station shaker or multi station magnetic stirrer to provide constant agitation of digestion flasks during enzymatic hydrolysis.
- (g) Beakers. Tall-form, 400 or 600 ml
- (h) Balance. Analytical, readability to 0.1 mg.

(i) pH meter Standardized with pH 7 and pH 4 buffers.

Reagents

(a) 95% Ethanol-v/v Technical grade.

(b) 78% Ethanol - Place 207 mL H₂O into 1 L volumetric flask. Dilute to volume with 95% ethyl alcohol. Mix and dilute to volume again with 95% ethyl alcohol if necessary. Mix One volume HO mixed with four volumes 95% ethyl alcohol will also give 78% ethyl alcohol final concentration.

(c) Acetone.

(d) Phosphate buffer. -0.08 M. pH 6.0. Dissolve 1.400 g sodium phosphate dibasic, anhydrous (Na₂HPO₄) (or 1.753 g dihydrate) and 9.68 g sodium phosphate monobasic monohydrate (NaH₂PO₄·H₂O) (or 10.94 g dihydrate) in ca 700 mL H₂O. Dilute to 1L with H₂O. Check pH with pH meter.

(e) α-amylase (heat stable). Termamyl.

(1) Store in refrigerator. Based on Nelson/Somogyi reducing sugar with soluble starch as substrate-10 000+1000 units/mL. (1 unit is defined as the amount of enzyme required to release 1 μmole reducing sugar equivalents/ min at pH 6.5 and 40°C).

(2) Based on Ceralpha method using p-nitrophenyl-maltosaccharide as substrate in the presence of α thermostable α-glucosidase- 3000 + 300 Ceralpha units/ml. (1 unit of enzyme is required to release 1 μmole p-nitrophenyl/min at pH 6.5 and 40°C)

(f) Protease-Keep refrigerated

(1) Casein assay- 300-400 Units/mL. [1 protease unit is defined as the amount of enzyme required to hydrolyze (and solubilize in TCA) 1 μmole tyrosine equivalents/min from soluble casein at pH 8.0 and 40°C]; 7-15 units/mg (1 unit will hydrolyze casein to produce color equivalent to 1.0 μmole tyrosine/min at pH 7.5 and 37°C). Color by Folin-Ciocalteu reagent (2) Azo-casein assay-300-400 Units/mL. 1 unit endo-peptidase activity defined as the amount of enzyme required to hydrolyze (and solubilize in TCA) 1 μmole tyrosine from soluble casein at pH 8.0 and 40°C]

(g) Amyloglucosidase-Keep refrigerated. (1) Strach / glucose oxidase -peroxidase 2000-3300 Units /mL (1 unit enzyme activity is defined as the amount of enzyme required to release 1 μmole glucose/ min at pH 4.5 and 40°C (p-nitrophenyl method- 130-200 Units/mL. [1 unit enzyme (PNP unit) is the amount of enzyme, which in the presence of excess levels of B-glucosidase, will release 1 μmole p-nitrophenyl from

p-nitrophenyl β -maltosidase/min at 40°C)

The only enzyme which has been found to be significantly contaminated with interfering activities is amyloglucosidase. Thermostable α -amylase and protease from commercial sources have been found to be generally free of interfering enzymes. Low levels of β -glucanase have been detected in protease preparations, at levels well below that which would interfere with total dietary fiber analysis. The major contaminant in amyloglucosidase endo-cellulase and resulted in oats, endo-depolymerization of mixed-linkage β -glucan from barley and oats, with resultant underestimation of this dietary fiber component. The contamination of amyloglucosidase with endo- cellulase (β -glucanase) can be easily detected.

Alternatively, there are kits containing all three enzymes (pretested) available from a number of companies.

(h) Sodium hydroxide solution -0.275 M. Dissolve 11.00 g NaOH ACS in ca 700 mL H₂O in 1 L volumetric flask. Dilute to volume with H₂O.

(i) Hydrochloric acid solution - 0.325 M. Dilute stock solution of known titer, e.g., 325 mL 1 M HCl, to 1 L with H₂O.

(j) Celite Acid-washed.

D. Enzyme Purity

To ensure absence of undesirable enzymatic activity in enzymes used in this procedure, run materials listed in through entire procedure each time lot of enzymes is changed, or at maximum interval of 6 months to ensure that enzymes have not degraded.

E. Test Portion Preparation

Determine total dietary fiber on dried test sample. Homogenize test sample and dry overnight in 70°C vacuum oven, cool in desiccator, and dry-mill test sample to 0.3-0.5 mm mesh. If test sample cannot be heated, freeze-dry before milling. If high fat content (>10%) prevents proper milling, defat with petroleum ether (3 times with 25 mL portions/g test sample) before milling.

Record loss of weight due to fat removal and make appropriate correction to final % dietary fiber found in determination. Store dry-milled test sample in capped jar in desiccator until analysis is carried out.

F. Determination

Run blank through entire procedure along with test portions to measure

any contribution from reagents to residue.

Weigh duplicate 1 g test portions, accurate to 0.1 mg. into 400 mL tall-form beakers. Test portion weights should not differ >20 mg. Add 50 mL pH 6.0 phosphate buffer to each beaker. Check pH and adjust to pH 6.0 ± 0.2 if necessary. Add 0.1 mL Termamyl solution. Cover beaker with Al foil and place in boiling water bath 15 min. Shake gently at 5 min intervals. Increase incubation time when number of beakers in boiling water bath makes it difficult for beaker contents to reach internal temperature of 95-100°C. Use thermometer to indicate that 15 min at 95-100°C is attained. Total of 30 min in water bath should be sufficient.

Cool solutions to room temperature. Adjust to pH 7.5 ± 0.2 by adding 10 mL 0.275 M NaOH solution.

Add 5 mg protease. [Protease sticks to spatula, so it may be preferable to prepare enzyme solution (50 mg in 1 mL phosphate buffer) and pipet 0.1 mL to each sample just before use]

Cover beaker with Al foil. Incubate 30 min at 60°C with continuous agitation. Cool. Add 10 mL 0.325 M HCl solution. Measure pH and dropwise add acid if necessary. Final pH should be 4.0-4.6. Add 0.3 mL amyloglucosidase, cover with Al foil, and incubate 30 min at 60°C with continuous agitation. Add 280 mL 95% ethyl alcohol preheated to 60°C (measure volume before heating). Let precipitate form at room temperature for 60 min.

Weigh crucible containing Celite to nearest 0.1 mg, then wet and redistribute bed of Celite in crucible by using stream of 78% ethyl alcohol from wash bottle. Apply suction to draw Celite onto fritted glass as even mat. Maintain suction and quantitatively transfer precipitate from enzyme digest to crucible.

Wash residue successively with three 20 mL portions of 78% ethyl alcohol, two 10 mL portions of 95% ethyl alcohol, and two 10 mL portions of acetone. Gum may form with some products, trapping liquid. If so, break surface film with spatula to improve filtration. Time for filtration and washing will vary from 0.1 to 6 h, averaging

0.5 h per sample. Long filtration times can be avoided by careful intermittent suction throughout filtration.

Dry crucible containing residue overnight in 70°C vacuum oven or 105°C air oven. Cool in desiccator and weigh to nearest 0.1 mg. Subtract crucible and Celite weight to determine weight of residue. Analyze residue from 1 test portion of set of duplicates for protein by using a conversion factor, except in cases where N content in protein is known,

Incinerate second test portion of duplicate 5 h at 525°C, Cool in desiccator and weigh to nearest 0.1 mg. Subtract crucible and Celite weight to determine ash.

G. Calculations

Determination of blank:

$$B = \text{blank, mg-weight residue} - P_B - A_B$$

where weight residue = average of residue weights (mg) for duplicate blank determinations; and P_B and A_B = weight (mg) of protein and ash, respectively, determined in first and second residues.

Calculate TDF as follows:

$$\text{TDF \%} = \left[\frac{\text{weight residue} - P - A - B}{\text{weight test portion}} \right] \times 100$$

SUGAR

where weight residue = average of weights (mg) for duplicate blank determinations, and P and A = weights (mg) of protein and ash respectively, in first and second test portion residues; and weight test portion = average of two test portion weights (mg)

PROCEDURE:

Weigh accurately about 5 g sample, transfer to a 200 ml volumetric flask dissolve in warm water, dilute to about 150 ml. In case solution is not clear, add 5 ml of Carrez 1 solution followed by 5 ml of Carrez 2 solution. Make up to 200 ml. Filter through a dry filter paper. Titrate the solution obtained as such to determine % Reducing sugars.

PRELIMINARY TITRATION:

Pipet 5 ml each of Fehling A and B into 250 ml conical flask. Mix and add about 10

ml water and a few boiling chips or glass beads. Dispense solution. Heat the flask to boiling. Add 3 drops of methylene blue indicator. Continue the addition of solution dropwise until the blue colour disappears to a brick-red end point. (The concentration of the sample solution should be such that the titre value is between 15 and 50 ml). Note down the titre value.

FINAL TITRATION:

Pipet 5 ml each of Fehling A and B. Add sample solution about 2 ml less than titre value of the preliminary titration. Heat the flask to boiling within 3 minutes and complete the titration. Perform the titration duplicate and take the average.

CALCULATIONS:

Reducing sugars (%) = Dilution x Factor of Fehling (in gm) / Weight of sample x Titre value *100

CALCIUM

Procedure:

Take a suitable aliquot of the sample solution prepared as prescribed in the material specification and expected to contain approximately 5 to 20 mg of calcium as CaO, in a 500 ml conical flask, Add 25 ml of triethanolamine solution, 10 ml of hydroxylamine hydrochloride solution and 2 ml of potassium cyanide solution. Dilute to about 150 to 200 ml with water and adjust the pH to 12.5 to 13 with 20 per cent sodium hydroxide solution using a pH indicator paper or preferably a pH meter. Add approximately 0.1 g of Patton and Reeder's indicator mixture and stir to dissolve. Titrate with 0.01 M EDTA solution from a burette, till the colour changes from red to pure blue free from any violet tinge. Towards the endpoint, the colour turns a violet which on further drop-wise addition gives the pure blue endpoint. It is easier to note the endpoint by comparing the colour with that of a previously titrated solution having a pure blue endpoint, where a slight excess of EDTA has been added.

Calculation:

Calcium (Ca) per cent by mass = $V_1 \times 0.04008 / M$

Where V_1 = Volume in ml of EDTX solution in titration; and

M = Mass in g of the sample in the solution for the test

IRON

Applicable to determination of Zn, Cu, and Fe in a variety of foods by dry ashing

and flame atomic absorption spectrometry (FAAS), and Cd and Pb by dry ashing and graphite furnace atomic Absorption spectrometry (GFAAS) Method is capable of determining these elements at concentrations above approximately Pb (0.3), Cd (0.1), Zn (1), Cu (5), and Fe (4) mg/kg.)

A. Principle

Test portions are dried and then ashed at 450°C under a gradual increase ($\leq 50^{\circ}\text{C}/\text{h}$ temperature. 6 M HCl (1+1) is added, and the solution is evaporated dryness. The residue is dissolved in 0.1 M HNO_3 , and the analytes are determined by flame and graphite furnace procedures.

B. Procedures

(a) Pretreatment - Homogenize product if necessary, using noncontaminating equipment. Check for leaching metals if the apparatus consists of metal parts.

(b) Drying-In a crucible, weigh 10-20 g test portion to nearest 0.01 g. Dry in a drying oven, on a water-bath, or a hot plate at 100°C, if there is a risk of heavy boiling in the ashing step. Proceed according to type of furnace.

(e) Ashing Ashing in a programmable furnace Place dish in furnace at initial temperature not higher than 100°C. Increase temperature at a maximum rate of 50°C/h to 450°C. Let dish stand for at least 8 h or overnight. Continue according to

(d) Ashing in a muffle furnace with thermostat following drying and pre-ashing in apparatus described in B(d)-(k)-See Figure 999.11. Place crucible with the test portion covered with the glass cover on the ceramic plate, and let purified air coming through a glass tube sweep over the product. Put IR lamp down at the cover Pre-ash product by increasing temperature slowly with IR lamp by gradually increasing temperature on hot plate to maximum. Final temperature on ceramic plate should then be about 300°C. Time required for pre-ashing varies with product. Put crucible in muffle furnace at 200-250°C and slowly raise

temperature to 450°C at a rate of no more than 50°C/h. Let stand for at least 8 h or overnight. Take crucible out of furnace and let cool.

(d) **Solution.** Wet ash with 1-3 ml. water and evaporate on water-bath or hot plate. Put crucible back in furnace at no more than 200°C and raise temperature (50-100°C/h) to 450°C. Proceed with ashing at 450°C for 1-2 h or longer. Repeat procedure until product is completely ashed, i.e., ash should be white/grey or slightly colored. Number of repetitions necessary varies depending on type of product. Add 5 ml. 6 M HCl, C(b), to crucible ensuring that all ash comes into contact with acid. Evaporate acid on water-bath or hot plate. Dissolve residue in 10.0-30.0 ml., to the nearest 0.1 ml, of 0.1 M HNO₃, C(d). Swirl crucible with care so that all ash comes

SODIUM AND POTASSIUM:

A. Apparatus and Reagents

(a) Glassware - Borosilicate glassware and intact Vycor, Pt or Si crucible precleaned with dilute HNO₃, and rinsed in distilled water immediately before use.

(b) Distilled water-H₂O, free from Na and K; either double-distilled or deionized. Use for preparing standards and dilutions.

(c) Sodium standard solutions - Stock solution-1 mg Na/mL. Dry reagent grade NaCl 2 h at 110°C; cool in desiccator. Weigh 2.5421 g into 1 L. volumetric flask and dilute to volume with H₂O. Working solution for flame emission -0.01, 0.05, and 0.05 mg Na/mL. Pipet 1, 3, and 5 mL. Na stock solution into separate 100 mL. volumetric flasks add 7 mL K stock solution and 2 mL HNO₃, to each flask dilute to volume with H₂O. Working solutions for flame absorption, -0.00003, 0.0001, 0.0003, and 0.0005 mg Na/mL. Pipet 1 mL. Na stock solution into 100 mL volumetric flask and dilute to volume with H₂O. Pipet 0.3, 1.0, 3.0 and 5.0 mL diluted stock solution into separate 100 mL. volumetric flasks and dilute to volume with H₂O

(d) Potassium standard solution- Stock solution- mg K/mL. Dry and cool reagent grade KCl as in c. Weigh 1.9068 g into 1 L volumetric flask and dilute to volume with H₂O. Working solutions for flame emission-0.04, 0.07, and 0.10 mg K/mL. Pipet 4, 7, and 10 mL. stock solution into separate 100 mL. volumetric flasks, add 3 mL. Na stock

solution to each flask and dilute to volume with H₂O. (5) Working solutions for flame absorption-0.0001, 0.0005, 0.0007, and 0.0010 mg K/mL. Pipet 1 mL K stock solution into 100 mL volumetric flask and dilute to volume with H₂O. Pipet 1, 5, 7, and 10 mL diluted stock solution into separate 100 mL volumetric flasks and dilute to volume with H₂O.

B. WET ASHING

Weigh 1 g test portion into 50 mL Pyrex beaker.

Dry 110°C, cool, and weigh if percent solids is to be determined

(a) Test samples with unknown or known high oil content Add ca 10 mL petroleum ether, and warm on steam bath or low temperature hot plate until oil is extracted. Decant and repeat until test portion is defatted.

(b) Test samples with low oil content- Add 5 mL HNO₃ [if total Cl content is desired, add enough 0.1 M AgNO₃ to precipitate chlorides (3.0 mL)] to each beaker. Digest on steam bath or low hot plate until test portion dissolves, evaporate to dryness. Add 5 mL HNO₃, and take to dryness. Repeat. Add 2 mL HNO₃, and warm to dissolved.

(c) For flame emission-Transfer digest to 25 mL volumetric flask with hot water, wash down sides of beaker three times with hot water, and add washings to flask. Cool, and dilute to volume with H₂O. If particles are too finely dispersed to settle, centrifuge aliquot at 2000 rpm.

(d) For flame absorption. Transfer digest to 100 mL volumetric flask and proceed as above. Dilute for direct readout as follows: Place 1 mL aliquot in 25 mL volumetric flask and dilute to volume with H₂O for Na; place 2 mL aliquot in 10 mL volumetric flask and dilute to volume with H₂O for K.

Prepare blank solution by diluting 2 mL HNO₃ to 100 mL with H₂O.

C. DRY ASHING

Weigh 4 g test portion into crucible, and char on electric hot plate or over low flame. Place in cold furnace and bring to 525°C. Ash 2 h to white ash. Cool, and weigh if total ash is desired.

Add 15 mL dilute HNO₃ (1+4) to crucible, breaking up ash with stirring rod if necessary. Filter through acid-washed quantitative paper into 100 mL volumetric flask. Wash residue and paper three times with H₂O. Dilute to volume. Proceed as in (a) or (b).

(a) For flame emission- Read directly.

(b) For flame absorption. Dilute for direct readout as follows: Place 1 mL aliquot in 100 mL volumetric flask and dilute to volume with H₂O for place 1 mL aliquot in 25 mL volumetric flask and dilute to volume with H₂O for K.

Prepare blank solution by diluting 2 mL HNO₃ to 100 mL with

D. DETERMINATION

Follow manufacturer's directions for type of instrument available. Dilute test solution if necessary to bring 7 readings within range of working standards. Read blank, standards, and test solutions at 589 nm for Na and 767 nm for K until results are reproducible; record percent T or percent absorption for each.

E. Calculations

For flame emission photometers not equipped with direct readout:

$$\text{mg Na or K/100g} = 100 \times F \left(\left[\frac{(E_x + E_1)}{(E - E_1)} \times (C_2 - C_1) \right] + C_1 \right) / \text{g test portion}$$

where (percent of unknown) - (percent T of blank) E_1 = percent T of standard of lower concentration than test solution - (percent T of blank) E_2 - (percent T of standard of higher concentration than test solution) - (percent T of blank); C_1 = mg Na or K/mL in standard of lower concentration than test solution C_2 = mg Na or K/mL in standard of higher concentration than test solutions F = dilution factor

For flame absorption photometers: Convert percent absorption to absorbance (A). Plot standard curve of A against concentration. Read unknown concentrations.

$$\text{mg Na or K/100g (concentration unknown} \times F \times 100) / \text{g test portion}$$

4.2.2 MICROBIOLOGICAL TEST

YEASTS AND MOULD

Micro-organisms which at 25°C form colonies in a selective medium

according to the method specified in this standard.

PRINCIPLE

Preparation of poured plates using a specified selective culture medium and a specified quantity of the test sample if the initial product is liquid, or of an initial suspension in the case of other products. Preparation of other plates, under the same conditions using decimal dilutions of the test sample or -of the initial suspension. Aerobic incubation of the plates at 25°C for 3,4 or 5 days. Calculation of the number of yeasts and moulds per gram or per milli litre of sample from the number of colonies obtained on plates chosen at dilution levels so as to give a significant result.

PROCEDURE

INOCULATION AND INCUBATION

Take two sterile petri dishes and Transfer to each dish, by means of a sterile pipette add 1ml of the test sample if liquid, or 1 ml of the initial suspension in the case of other products. Take two further sterile petri dishes. Transfer, by means of another sterile pipette, 1 ml of the 10^{-1} dilution to each dish (liquid product), or 1 ml of the 10^{-2} dilution (other product). Repeat the procedure described above using further dilutions, if necessary. Pour about 15 ml of the yeast extract dextrose chloramphenicol-agar medium previously melted and maintained at $45\pm 1^{\circ}\text{C}$ a water-bath from a culture bottle into each petri dish. The time elapsing between the end of the preparation of the initial suspension (or of the 10^{-1} dilution if the product is liquid) and the moment when the medium is poured into the dishes shall not exceed 15 min. Carefully mix the inoculum with the medium and allow the mixture to solidify, by leaving the petri dishes to stand on a cool -horizontal surface. Prepare a control plate, with 15 ml of the medium, to check its sterility. Invert the plates and place them in the incubator at $25 \pm 1^{\circ}\text{C}$.

PLATE COUNT

yeast and mould-forming countable colony, produced under the conditions specified in this International Standard

PRINCIPLE

- Two poured plates are prepared using a specified culture medium and a specified quantity of the test sample, if the initial product is liquid, or using a specified quantity of an initial suspension in the case of other products. Other

pairs of poured plates are prepared, under the same conditions, using decimal dilutions of the test sample or of the initial suspension.

- The plates are aerobically incubated at 30 °C for 72 h.
- The number of microorganisms per millilitre or per gram of sample is calculated from the number of colonies obtained on selected plates.

PROCEDURE

Test portion, initial suspension and dilutions See the relevant part of ISO 6887 and the specific International Standard dealing with the product concerned.

INOCULATION AND INCUBATION

- Take two sterile Petri dishes and Transfer to each dish, by means of a sterile pipette 1 ml of the test sample, if liquid, or 1 ml of the initial suspension in the case of other products (10–1 dilution).
- Take two other sterile Petri dishes Transfer to each dish, by means of another sterile pipette, 1 ml of the 10–1 dilution (liquid product) or 1 ml of the 10–2 dilution (other products).
- If necessary, repeat the procedure with the further dilutions, using a new sterile pipette for each decimal dilution.
- If appropriate and possible, select only the critical dilutions steps (at least two consecutive decimal dilutions) for the inoculation of the Petri dishes that will give colony counts of between 15 and 300 colonies per plate.
- Pour about 12 ml to 15 ml of the plate count agar at 44 °C to 47 °C into each Petri dish. The time elapsing between the end of the preparation of the initial suspension (or of the 10–1 dilution if the product is liquid) and the moment when the medium is poured into the dishes shall not exceed 45 min.
- Carefully mix the inoculum with the medium by rotating the Petri dishes and allow the mixture to solidify by leaving the Petri dishes standing on a cool horizontal surface. After complete solidification, and only in the case where it is suspected that the product under examination contains microorganisms whose colonies will overgrow the surface of the medium, pour about 4 ml of the overlay medium at 44 °C to 47 °C onto the surface of the inoculated medium.

- Allow to solidify as described above. 9.2.8 Invert the prepared dishes and place them in the incubator at $30\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for $72\text{ h} \pm 3\text{ h}$. Do not stack the dishes more than six high. Stacks of dishes should be separated from one another and from the walls and top of the incubator.

Counting of colonies

- After the specified incubation period, count the colonies on the plates, using the colonycounting equipment if necessary. Examine the dishes under subdued light. It is important that pinpoint colonies should be included in the count, but it is essential that the operator avoid mistaking particles of undissolved or precipitated matter in dishes for pinpoint colonies. Examine doubtful objects carefully, using higher magnification where required, in order to distinguish colonies from foreign matter.
- Spreading colonies shall be considered as single colonies. If less than one-quarter of the dish is overgrown by spreading, count the colonies on the unaffected part of the dish and calculate the corresponding number of the entire dish. If more than one-quarter is overgrown by spreading colonies, discard the count.

COLIFORMS:

Bacteria which, at the specified temperature (i.e. $30\text{ }^{\circ}\text{C}$, $35\text{ }^{\circ}\text{C}$ or $37\text{ }^{\circ}\text{C}$, as agreed) form characteristic colonies in crystal violet neutral red bile lactose agar under the test conditions specified in this International Standard.

PRINCIPLE

- Preparation of two poured plates, using a solid selective culture medium, and using a specified quantity of the test sample if the initial product is liquid, or using a specified quantity of an initial suspension in the case of other products.
- Preparation of other pairs of poured plates, under the same conditions, using decimal dilutions of the test sample or of the initial suspension. incubation of the plates at $30\text{ }^{\circ}\text{C}$, $35\text{ }^{\circ}\text{C}$ or $37\text{ }^{\circ}\text{C}$ (as agreed) for 24 h.
- Calculation of the number of coliforms per millilitre or per gram of sample from the number of characteristic colonies obtained in the plates.\

PROCEDURE

Test portion, Initial suspension and dilutions See ISO 6887 and the specific

International Standard appropriate to the product concerned.

INOCULATION AND INCUBATION

- Take two sterile Petri dishes and Using a sterile pipette (6.4), transfer to each dish 1 ml of the test sample, if the product is liquid, or 1 ml of the initial suspension In the case of other products.
 - Take two other sterile Petri dishes. Using a fresh sterile pipette, transfer to each dish 1 ml of the first decimal dilution (10^{-1}) of the test sample, if the product is liquid, or 1 ml of the first decimal dilution (10^{-2}) of the Initial suspension in the case of other products.
 - Repeat the procedure described with the further dilutions, using a fresh sterile pipette for each decimal dilution.
 - Pour about 15 ml of the VRBL medium at $45^{\circ}\text{C} \pm 0,5^{\circ}\text{C}$, into each Petri dish. The time elapsing between the end of the preparation of the initial suspension (or of the 10^{-1} dilution if the product is liquid) and the moment when the medium is poured into the dishes shall not exceed 15 min.
 - Carefully mix the inoculum with the medium and allow the mixture to solidify, with the Petri dishes standing on a cool horizontal surface. Also prepare a control plate, with 15 ml of the medium for checking its sterility.
- 9.2.3 After complete solidification, pour about 4 ml of the VRBL medium at $45^{\circ}\text{C} \pm 0,5^{\circ}\text{C}$, on to the surface of the inoculated medium. Allow to solidify as described above.
- Invert the prepared dishes and incubate them in the incubator set at 30°C , 35°C or 37°C (as agreed) for $24\text{ h} \pm 2\text{ h}$

ESCHERICHIA COLI

The typical *Escherichia coli* is aerobic, Gram-negative rod, motile, fermenting lactose with the production of gas and usually produces smooth, non-mucoid colonies on solid media. However, there are non-lactose fermenting strains of *Escherichia coli* and some strains produce mucoid colonies. The organism also exhibits the following characters:

- a. Hydrogen sulphide (H_2S) production when done in TSI medium: Negative
- b. Urease: Negative
- c. Indole: Positive

- d. Methyl red: Positive
- e. Voges-Proskauer test: Negative
- f. Simmon's citrate: Not utilized
- g. Sucrose: Acid and gas production variable
- h. Salicin: Acid and gas production variable, and
- i. Growth at 44°C in MacConkey broth medium: Positive with acid and gas.

PROCEDURE FOR ISOLATION

blend the sample in a sterile blender jar for 2 minutes or macerate--with sterile sand in a sterile mortar using approximately 200 ml of diluting fluid per 25 g of the sample. The diluting fluid for preparing the homogenate should be 1 percent peptone solution in water, sterilized at 120°C for 20 minutes and final pH adjusted 6.8 $\pm$ 0.1, or 3-4 percent of potassium dihydrogen phosphate (KH₂PO₄) in water, pH adjusted to and sterilized at 120°C for 20 minutes. Inoculate 1 ml of the blended or macerated sample into 10 ml of single strength MacConkey broth medium. If the number of organisms are assumed to be very small, inoculate 10 ml of double strength MacConkey broth medium Also streak loopfuls on to MacConkey agar medium , eosin methylene blue lactose agar , and if available Tergitol-7 agar Incubate all the inoculated media at 37°C overnight. If there is growth with fermentation of lactose in the MacConkey broth medium streak out a loopful on to each of the solid media and incubate at 37°C overnight.

SALMONELLA

Microorganisms which form typical colonies on solid selective media and which display the biochemical and serological characteristics described when tests are carried out in accordance with this International Standard

DETECTION OF SALMONELLA:

Determination of the presence or absence of these microorganisms, in a particular mass of product, when tests are carried out in accordance with this International Standard.

PRINCIPLE

The detection of Salmonella necessitates four successive stages

Salmonella may be present in small numbers and are often accompanied by considerably larger numbers of other members of Enterbacteriaceae or of other families. Therefore, selective enrichment is necessary; furthermore, pre-enrichment is often necessary to permit detection of injured Salmonella.

PROCEDURE

Test portion and initial suspension

- For preparation of the initial suspension, use as dilution fluid the pre-enrichment medium specified.
- In general, to prepare the initial suspension, add a 25 g test portion to 225 ml of pm-enrichment medium which is the ratio of test portion to pre-enrichment medium specified in this method.
- If the prescribed test portion is other than 25 g, use the necessary quantity of pre-enrichment medium to yield approximately a 1/10 dilution (mass to volume).

4.2.3 SENSORY EVALUATION OF COOKIES

Sensory evaluation of quinoa millet cookies was carried on 5point hedonic scale. The average scores of the 40 judges for different quality characteristics. appearance, smell, taste, touch, hear and overall acceptability were recorded.

CHAPTER-5

RESULT AND DISCUSSION

The experimental bakery products, developed in the present study using refined wheat, flax seed, multigrain quinoa millets, were characterized to ensure acceptability and edibility for both the general population and elderly individuals, incorporating various parameters nutritional analysis, microbial analysis and sensory evaluation for product development.

5.1 NUTRITIONAL EVALUATION OF QUINOA MILLET COOKIES:

S.NO	PARAMETERS	UNITS	RESULTS
1	Energy	KCAL/100g	495
2	Carbohydrate	g/100g	65.9
3	Total fat	g/100g	21.5
4	Protein	g/100g	9.54
5	Dietary fibre	g/100g	6.18
6	Total sugar	g/100g	21.9
7	Sodium as Na	mg/100g	204
8	Potassium as K	mg/100g	256
9	Calcium as Ca	mg/100g	102
10	Iron as Fe	mg/100g	3.74

TABLE5.1: NUTRITIONAL CONTENT OF THE QUINOA MILLET BISCUIT

The data table 1 provides a comprehensive overview of the nutritional composition of quinoa millet biscuits across various parameters. Analysis indicates that these biscuits contain per 100g 495 Kcal for energy, 65.9g for of carbohydrates, 21.5 g for total fat, 9.54 g per for protein, 6.18 g per for fiber, 21.9 g for total sugar, 204 mg for sodium, 256 mg for potassium, 102 mg 100 g for calcium, and 3.74 mg for iron. These values reflect the nutritional richness and potential health benefits of incorporating quinoa millet biscuits into one's diet. The quinoa millet biscuit, providing 3.74 mg of iron, helps alleviate anemia in patients while meeting the daily iron requirement of 10-20 mg in a balanced diet. Sedentary women or men with a

recommended dietary allowance (RDA) of 1645 kcal benefit from its 495 kcal energy contribution.

5.2 MICROBIOLOGICAL EVALUATION OF QUINOA MILLET COOKIES:

S.NO	PARAMETERS	UNITS	RESULTS
1	TOTAL PLATE COUNT	CFU/g	1200
2	YEAST& MOULD COUNT	CFU/g	<10
3	COLIFORM COUNT	CFU/g	<10
4	ESCHERICHIA COLI	CFU/g	<10
5	SALMONELLA SPP	Per 25 g	ABSENT

TABLE5.2: MICROBIOLOGICAL CONTENT OF THE QUINOA MILLET BISCUIT

This data table 2 presents a detailed microbiological analysis of quinoa millet biscuits. The analysis reveals that these biscuits have a total plant count of 1200 CFU/g, with yeast and mold counts below 10 CFU/g, and coliforms and *Escherichia coli* counts also below 10 CFU/g. Moreover, the absence of *Salmonella spp* per 25 g further underscores the safety and quality of these biscuits. This comprehensive overview assures consumers of the high standard of hygiene and microbiological safety maintained in the production of quinoa millet biscuits.

5.3 SENSORY EVALUATION OF BISCUIT:

S.NO	SENSORY	5 ☆	4☆	3☆	2 ☆	1 ☆	OVERALL RATING
1	Appearance	30	9		1		4.7
2	Smell	33	4		2	1	4.6
3	Taste	25	6	3	5	1	4.2
4	Touch	25	12	3			4.5
5	Hear	23	10	6	1		4.4

OVER ALL ACCEPTABILITY = 4.5

TABLE5.3: SENSORY EVALUATION CONTENT OF THE QUINOA MILLET

BISCUIT

A panel of 40 individuals from diverse age groups was assembled to conduct a sensorial evaluation of quinoa millet biscuits enriched with hemp flour. Using a 5-point Hedonic scale, panel members rated various attributes including appearance, flavor, smell, touch, and overall taste. While the biscuits received lower scores for appearance (4.7), smell (4.6), touch (4.5), and sound (4.4) due to the altered appearance caused by the addition of hemp flour, they garnered higher scores for flavor and taste (over acceptability 4.5). Consumers appreciated the distinctive notes of quinoa millet, contributing to a balanced sweet-aromatic-slightly sour taste profile. Despite the initial lower ratings, the product was easily accepted by consumers, as evidenced by the overall weighted average grade of 4.5. Additionally, it's noted that a simple modification, such as changing the shape to a circle, could potentially improve the overall acceptance of the product further.

PACKING AND LABELLING:





Fig 5.1 PACKING AND LABELLING

CHAPTER-6

SUMMARY AND CONCLUTION:

The study concluded that developing and standardizing baked product recipes using quinoa millets and flaxseed as substitutes for maida flour holds immense potential. Quinoa millets, rich in minerals like iron and calcium, along with other functional components, offer numerous health benefits and can help address various nutrition-related disorders such as deficiencies, diabetes, and obesity. Although quinoa millet-based products may have lower shelf stability, this issue can be significantly mitigated by pre-treating the grains before milling and storing them refrigerated, as suggested in the study.

Quinoa millet-based snacks could serve as healthier alternatives to popular but less nutritious junk foods currently dominating the market. Biscuits, being convenient carriers of nutrients, are particularly suitable for delivering the abundant health benefits of quinoa millet to people of all age groups. Moreover, these biscuits are not only economical and shelf-stable but also highly acceptable among consumers. The salted and sweet biscuits developed using 95% quinoa millet flour in the study are not only high in energy and protein density but also rich in essential minerals like iron, calcium, and phosphorus. Commercializing quinoa millet products can offer a healthy alternative for consumers with special dietary needs such as anemia, diabetes, celiac disease, pregnant and lactating mothers, children, adolescents, and individuals with dementia. Integrating these products into schemes like Integrated Child Development Services (ICDS) can help address protein-energy malnutrition and anemia prevalent in many developing countries.

Furthermore, the study found that baked products, when stored in airtight glass jars at room temperature, exhibit good storage stability, with quinoa millet biscuits having a maximum shelf life of one week.

REFERENCES

1. B.srilakshmi. (2021). *dietetics*. new delhi: new age international(P)limited.
2. Bhargava, A. e. (2006). Chenopodium quinoa – An Indian perspective. *Ind Crops Prod*, 73–87.
3. Charu Katare *et al.*, (2012). Flax Seed: A Potential Medicinal Food.
4. Colombel JF, S. A. (2019 Feb). AGA Clinical Practice Update on Functional Gastrointestinal Symptoms in Patients With Inflammatory Bowel Disease:. *[PubMed]*, 380-390.
5. Cunnane *et al.*, (1993). High-linolenic acid flaxseed(*Linum usitatissimum*)some nutritional properties in humans. *British Journal of Nutrition*.69, 443-453.
6. Daraz. O *et al.*, (2020). Development of Quinoa (*CHENOPODIUM QUINOA*) supplemented cookies. *Agric. Sci. J.* 2(1):, 56-66.
7. DeMaeyer EM, D. P. (1989). Preventing and Controlling Iron Deficiency Anemia through Primary Health AARE:. *A Guide for Health Administrators and Programme Managers*. Geneva: *World Health Organization*; , 1-61.
8. Dharendra Kumar Dinkar *et al.*, (2020). Preparation of Biscuits by Using Lotus Seed, Pearl Millets and Multigrain Wheat for Elderly People. *International Journal of Research in Engineering, Science and Management*, Volume-3 ,413-415.
9. Dipankar Maiti *et al.*, (2021). Iron deficiency anemia- An updated overview. *International Journal of Research in Pharmacy and Pharmaceutical Sciences*, 28-33.
10. Dmochowska N, W. H. (2018 Aug). Advances in Imaging Specific Mediators of Inflammatory Bowel Disease. *[PubMed]*, 9.
11. Dorna Davani-Davari *et al.*, (09 march 2019). Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications.
12. Feuerstein JD, P. K.-V. (2018 Jan). Targeted Physician Education and Standardizing Documentation Improves Documented Reporting with Inflammatory Bowel Disease. *[PubMed]*, :36-45.
13. Gallo G, K. P. (2018 Feb). Surgery in ulcerative colitis. *[PubMed]*, 71-78.
14. Gamal Saad Elhadidy *et al.*, (july 2020). Production of Biscuits High Nutritional Value . *Food Technology Research Institute* , 1569-1578.
15. Khaled M *et al.*, (2017). Chemical Characteristic and Sensory Evaluation of Biscuit. *Enriched with Wheat Germ and the Effect of Storage Time on the Sensory Properties for this Product*.
16. Kucuk, O. (2002). New opportunities in chemoprevention. *Cancer Investigation*, 237-245.
17. Maaser C, S. A.-L. (2019 Feb 01;13). Guideline for Diagnostic Assessment in IBD Part 1: Initial diagnosis, monitoring of known IBD, detection of complications. *J Crohns Colitis*. . *[PubMed]* , (2):144-164.
18. Mariane Lutz *et al.*, (2017). The Revival of Quinoa: A Crop for Health.
19. Maxwell Chufama, (2021,). An Understanding of the Development of Marketing, Consumer Behaviour Place in Marketing and Consumer Behavior Notions. *International Journal of Multidisciplinary Research and Publications*, 9-17.
20. Mitrev N, V. C.-S. (2017 Dec). IBD Sydney Organisation and the Australian Inflammatory Bowel Diseases Consensus Working Group. Review article: consensus statements on therapeutic drug monitoring of anti-tumour

- necrosis factor therapy in inflammatory bowel diseases. . *[PubMed]*, 1037-1053.
21. Mujica A. (1994). Andean grains and legumes, in *Neglected Crops*:. *Hernando Bermujo JE, Leon J. FAO, Rome, pp, 131-148.*
 22. Nguyen VQ, M. J. (2018 Jan). Knowledge Gaps in the Management of Postoperative Crohn's Disease. *PubMed*, 53-60.
 23. Nihir Soni *et al*, (2018). Studies on development of high protein cookies . *International Journal of Chemical Studies* .
 24. Pariente B, H. S. (2019 Apr). Treatments for Crohn's Disease-Associated Bowel Damage: . *[PubMed]*, 847-856.
 25. Park K, e. (2019). *Park's Textbook of Preventive and Social Medicine*. . Jabalpur:: Banarsidas Bhanot Publishers; .
 26. PP Salunke *et al.*, (30 june 2019). Studies on nutritional quality of barnyard millet . *Department of Food Science and technology*, 651-657.
 27. Prolay Paul *et al.*, (08 august 2021). Iron deficiency anemia. *International Journal of Research in Pharmacy and Pharmaceutical Sciences*, 28-33.
 28. Prolay Paul *et al.*, (2021). Iron deficiency anemia- An updated overview. *International Journal of Research in Pharmacy and Pharmaceutical Sciences*, 28-33.
 29. Rosna Tadju *et al.*, (2021). Sensory evaluation of panelists on biscuit products based on sorghum flour and corn bran flour.
 30. Sargun Kaur *et al.*, (146-153). Marketing Flows and Consumer Behavior: An Analysis.
 31. Shashi Bhushan Kumar *et al.*, (20 july 2022). Iron Deficiency Anemia. *Efficacy and Limitations of Nutritional and Comprehensive Mitigation Strategies*, 1-20.
 32. Shekhara Naik R *et al.*, (08 july 2020). Flax Seeds (*Linum usitatissimum*): Nutritional composition and health benefits. *IP Journal of Nutrition, Metabolism and Health Science*, 35-40.
 33. Smita Kagwad *et al.*, (June 2019). A STUDY ON IMPACT OF BAKING INDUSTRY IN ECONOMY AND GROWTH OF ORGANIC BAKING. *Journal of Emerging Technologies and Innovative Research (JETIR)*, Volume 6 ,870-879.
 34. Soccol *et al.*, (2003). Seafood as Seafood as. *Brazilian Archives of Biology and Technology*,, 443-454.
 35. Sturm A, M. C. (2019 Mar). European Society of Gastrointestinal and Abdominal Radiology. *PubMed*, :273-284.
 36. Su HJ, C. Y. (2019 Jul). Inflammatory bowel disease and its treatment in. *[PubMed]*, 118(7):1083-1092.
 37. Sunita Mishra, .. (2020). Preparation of Biscuits by Using Lotus Seed, Pearl Millets and Multigrain Wheat Preparation of Biscuits by Using Lotus Seed, Pearl Millets and Multigrain Wheat Preparation of Biscuits by Using Lotus Seed, Pearl Millets and Multigrain Wheat. *International Journal of Research in Engineering, Science and Management*, 2581-5792.
 38. susan.f clark. (2018). Iron Deficiency Anemia. *nutrition in clinical practice*, 128-141.
 39. Thaisa Menezes Alves Moro *et al.* (2018). Use of burdock root flour as a prebiotic ingredient in cookies. 540-546.
 40. Trivedi PJ, A. D. (2018 Aug). Chemokines and Chemokine Receptors as Therapeutic Targets in Inflammatory Bowel Disease; . *[PubMed]*, S641-

S652.

41. Vandana *et al.*, (2020). Formulation of quinoa (*Chenopodium quinoa*) biscuits and evaluation of its physical, textural, sensory and nutritional quality. *Journal of Pharmacognosy and Phytochemistry*.
42. Viktoria Angeli *et al.*, (19 february 2020). Quinoa (*Chenopodium quinoa* Willd.): An Overview of the Potentials of the “Golden Grain” and Socio-Economic and Environmental Aspects of Its Cultivation and Marketization.
43. West AR, O. P. (2008;). Mechanisms of heme iron absorption: current questions and controversies. *World J Gastroenterol*, 14: 4101-4110.
44. Zevallos *et al.*, (2015). Gastrointestinal effects of eating quinoa (*Chenopodium quinoa* Willd.) in celiac patients. *American J. Gastroenterol.* 109:, 270-278.
45. Zhang, L. e. (2018). Recent Developments in Functional Bakery Products and the Impact of Baking on Active Ingredients. *21st International Drying Symposium València*,, 667-674.