DEVELOPMENT OF VALUE-ADDED GLUTEN-FREE COOKIES FOR GLUTEN-SENSITIVE DISEASE

AARTHI SWETHA.M

(21PFD001)

Thesis submitted to

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore - 641043

In Partial Fulfillment of the Requirements for the

Degree of Master of Science in

FOOD SERVICE MANAGEMENT AND DIETETICS

MAY, 2023

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Signature of the Supervisor

Signature of the Head of the Department

CERTIFICATE

This is to certify that the thesis entitled, **"Development of value-added gluten-free cookies for gluten-sensitive disease"** submitted to Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore in partial fulfillment of the requirements for the award of the degree of Master of Science in Food Service Management and Dietetics , is a record of original research work done by **Ms. M.Aarthi Swetha** with Register Number 21PFD001 during the period of this study under the Supervision and Guidance of **Dr.V.Premala priyadharshini, Professor and Head of the Department** of Food Service Management and Dietetics, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore – 641 043, Tamil Nadu , India.

Signature of the Supervisor

Signature of the Candidate

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INTRODUCTION

I INTRODUCTION

Food Allergy affects roughly 8% of children in Western nations and appears to be spreading to other regions of the world, notably metropolitan areas as opposed to rural ones, including Vietnam and South Africa and Asia. The prevalence of food allergies in both adults and children is now estimated to be around 10%, suggesting that the incidence of food allergies has been rising globally in recent years (Gupta RS et al., 2019).One of the top five foods that frequently causes allergic reactions in children is wheat(Srisuwatchari W et al.,2022). In the first three years of life, roughly 6% of children have food allergies, including about 2.5% of people who have allergies to cow's milk, 1.5% to eggs, and 1% to peanuts (El-Sayed, Z., & Shousha, G. 2020)

Cereals like wheat (gliadins and glutenins), rye (secalins and secalinins), oat (avenins and avenalins), and barley (hordeins and hordenins) all contain gluten, a protein molecule. The ethanol-soluble proteins that comprise gluten are prolamins and glutelins. These proteins are abundant in glutamine and proline residues and are not broken down by human intestinal proteases, giving the dough the flexibility required for leavening and shaping.(Caio, G. et al., 2019)(Bascunan, K.A et al.,2020)Two wheat proteins, gliadins and glutenins, have been linked to allergic reactions in susceptible individuals, leading to wheat allergies. (Shewry, P. 2023).

According to Yoosuf, S., and Makharia, G. K. (2019), the gluten prolamins—also known as gliadins in wheat, secalins in rye, a combination of both in triticale, hordeins in barley, and avenins in oats—have been associated to Celiac Disease. Environmental elements including early wheat exposure and ingesting processed wheat products may also have an impact on the development of wheat allergy (Sabença, C., et al., 2021).

Changes in the gut microbiota, which can be impacted by factors like nutrition and antibiotic use, may alter the immune system's response to wheat proteins.(Garca-Montero et al., 2021).A mild or severe allergic reaction to foods containing wheat is known as a gluten allergy. It may manifest as digestive tract symptoms, nasal congestion, urticaria, skin rashes, congestion, throat irritation, coughing, or breathing problems (Ricci G et al., 2019). It is distinguished by an immune response to glutenin and gliadin, two proteins found in wheat, and it can result in a range of symptoms, from minor hives and itching to life-threatening anaphylaxis. (N. Nadpara et al.,2022).IgE and non-IgE pathways are both involved in wheat allergies. The typical duration of IgE-mediated wheat allergy symptoms is between minutes and two hours following wheat consumption. (W. Srisuwatchari., et al., 2022)

Celiac disease ,wheat allergy and non-celiac gluten sensitivity are the most common Gluten-related disorders(Al-Toma et al ., 2019).According to Sergi, et al., 2021 roughly 3% of people worldwide experience wheat intolerance (1% with Wheat Allergy,1% with celiac disease, and 1% with non-celiac gluten sensitivity).In the United States, It is estimated that 0.71 percentage of adults have celiac disease(Aboulaghras, S., et al.,2023). According to the serological findings, celiac disease morbidity ranges from 1.1 % to 1.7% globally. The Asia-Pacific area has an increasing prevalence of celiac disease, with rates varying from 0.1% to 1.5% depending on the nation. (Pourhoseingholi, M. A. 2022)

The prevalence rate of non-celiac gluten sensitivity is between 0.6% and 10.6%.From infants to the elderly, anyone can get celiac disease at any age. In particular, if they have a family history of the condition or other autoimmune conditions, adults may also be diagnosed with celiac disease. The condition can manifest in two distinct peaks, the first occurring within the first two years of life after gluten weaning and the second occurring in the second or third decades of life (Caio, G., et al., 2019). Most adults in their third or fourth decade of age will have or exhibit non-celiac gluten sensitivity.

According to Black, C. J., & Ford, A. C. 2020 Females tend to be more prone towards having Non Celiac Gluten-sensitivity (NCGS) when compared to that males. The ratio is said to be (3:1-5.4:1). Survey research indicates that Non Celiac Gluten-Sensitivity (NCGS) prevalence rates range from 0.49% to 14.9%, which are greater than almost all estimates of Celiac disease or Wheat allergy prevalence rates.(Ponzo, V et al.,2021). According to Cabanillas.,2020 Gluten sensitivity may be on the rise among children and teenagers. According to Volta, U., et al. 2019, adults with certain medical conditions, such as irritable bowel syndrome (IBS) and autoimmune diseases, have higher rates of gluten sensitivity than the general population, which is thought to be around 6% (Koloski, N. et al., 2019)

Celiac disease has a strong hereditary correlation, including polymorphisms in the HLA-DQ genes. Some mutations of these genes, which are important for the way the immune system reacts to gluten, could render people more likely to develop the celiac disease (Sallese, M., et al .,2020)The development of celiac disease may also be influenced by environmental factors (Serena, G., et al .,2019). A reaction to other proteins in wheat, such as amylase trypsin inhibitors (ATIs), maybe the cause of gluten sensitivity. Increased intestinal permeability, commonly known as a leaky gut syndrome, or an imbalance in the gut flora can also contribute to gluten sensitivity. Gluten sensitivity may be influenced by both genetic and environmental factors. Gluten sensitivity can be caused by changes in the expression of genes related to the intestinal barrier function and immune system

dysregulation (Caio, G.,et al.,2020).Stress and other environmental elements have also been mentioned as potential gluten-sensitivity .triggers(Mumolo, M. G.,et al.,2020).Gluten sensitivity has been linked to other illnesses like fibromyalgia, chronic fatigue syndrome, and irritable bowel syndrome.(Volta, U.,et al.,2019)Several factors, such as stress, infections, modifications to the gut flora, and hormonal changes, might cause the development of gluten sensitivity.(Akobeng, A. K.,et al.,2020) .Wheat substances other than gluten, like FODMAPs and trypsin and amylase inhibitors (ATIs), could also serve as triggers for various clinical symptoms in NCGS cases, whether they be intestinal, extraintestinal or both. Wheat amylase and trypsin inhibitors, complex proteins that increase innate immunity, may also increase NCGS symptoms(Cárdenas-Torres, F. I et al.,2021)

Intolerance of Gluten-a protein found in cereals like wheat, barley, rye, and oats causes the pathogenic process. When gluten-intolerant person consumes food containing gluten the host immune system is self-activated and produces "anti-gliadin" antibodies that are produced in the intestine, triggering an inflammatory response in the mucosa(Stoin, D., et al.,2021) Celiac disease (CD) develops when individuals with certain predisposing genes get exposed to gluten-containing cereals (wheat, barley, or rye) celiac a T-cell-mediated autoimmune disorder. Prolamins in wheat, rye, and barley are the main source of individuals' digestive issues for those who have celiac illnesses(Di Liberto, D et al., 2020). The onset of celiac disease may be driven by factors such as age at the time gluten was first introduced to the diet, viral infections, and alterations to the gut microbiota (Leonard, M. M., et al.,2020)

Irrespective of children and adults the common gastro intestinal symptoms of gluten sensitive disease includes bloating, abdominal pain, diarrhoea, nausea and reflux, whereas the extra-intestinal signs are extremely diverse and they include depression, dermatitis, fibromyalgia, generalized fatigue, headaches, and mental fogginess. (Cárdenas-Torres, F.I et al.,2021).

In the case of wheat allergy, young children are more likely to experience gastroenterological symptoms including vomiting, diarrhoea, or, less frequently, abdominal pain. In roughly 40% of children skin complaints in the form of urticaria, erythema, angioedema, pruritus, or at worse autonomic dysreflexia (AD) can be seen. Children affected with wheat allergy (WA) will mostly be affected by other food and non-food allergies like AD (78%–87%). Asthma and/or Rheumatoid Arthritis (RA) affect 50% of individuals (range: 48%–67%) (El-Sayed, Z., & Shousha, G.,2020).

Only one in eight patients with Celiac Diease (CD) is believed to have had a good diagnosis after exhibiting apparent symptoms, suggesting that many more people may be misdiagnosed as a result of unrecognised (non-specific) symptoms. This is known as latent and silent CD. (Brouns, F., et

al.,2019). For diagnosis, Patients who are suspected of having a gluten-related disease should first go through an in-depth clinical and laboratory evaluation to rule out Celiac Disease (CD) and Wheat Allergy (WA) while still consuming gluten.

The patient should then consume gluten-containing foods regularly for at least six weeks. Although the pathophysiology of Non Celiac Gluten - Sensitivity (NCGS) is uncertain, findings indicate that the innate immune system plays a major role in the disease. (Shahbazkhani, B et al.,2020) .Since there is no sensitive and distinct biomarkers for NCGS diagnosis, it is challenging to pinpoint incidences of the disease. Only a few of the several biomarkers that have been proposed so far for the diagnosis of Non Celiac Gluten - Sensitivity (NCGS) include the analysis of eosinophils, intraepithelial CD3+ T cells, T helper lymphocytes, mast cells, cytokine and antibody serum levels, RNA transcripts, and miRNA signatures. Even though these biomarkers are not uniquely associated with NCGS, the fact that they may be distinguished from other disorders by their use as clinical laboratory parameters allow for the identification of potential Non Celiac Gluten - Sensitivity (NCGS) cases.(Masaebi, F. et al.,2020)

Gluten-free diets are the best ways to treat gluten sensitivity (Bahaciu, G.V. et al.,2022). For those with Celiac Disease (CD), the recommended course of treatment is a lifelong gluten-free diet (GFD)(Aljada, B., et al.,2021). Utilizing different Gluten free flours such as rice, maize, and sorghum, pseudocereals such as quinoa, amaranth, and buckwheat, legumes such as soy, chickpea, and carob germ flour, starches such as corn, potato, cassava, modified starch, fruits, vegetables, and milk can help to overcome this problem.(Mohammadi, M.,et al.,2022).

Pseudocereal grains are dicotyledonous species of edible seeds that are characterised as such because of their high starch content and morphological similarity to real cereals (monocotyledonous of the Poaceae family). They are nutrient-dense grains that are gluten-free (GF) and have substantial nutraceutical potential. They contain high concentrations of carbohydrates, fibre, and proteins of superior quality with a balanced composition of necessary amino acids. They also contain considerable amounts of amino acids rich in sulphur. They also provide a good amount of minerals, phytochemicals, and vitamins. (Martnez-Villaluenga, C., et al, 2020).

The gluten-free flour that is most frequently used to make gluten-free products is rice flour, which is frequently combined with other plant-based ingredients such as waxy rice starch, maize starch and pea protein, and maize starch/flour and potato starch/flourto get the best batter or dough characteristics and bakery product quality.Most gluten-free flours, including those made from rice,

maize, sorghum, buckwheat, quinoa, lupin, chickpea, and others, are used to make gluten-free cookies, biscuits, cakes, muffins, and crackers.

Chemically leavened gluten-free products, in particular cookies, biscuits, cakes, muffins, and crackers, make up a significant amount of bakery goods in addition to bread, noodles, and pasta. Celiac disease patients tend to consume more gluten-free crackers and cookies than gluten-free bread. Pinto bean flour, buckwheat flour and alfalfa seed flour, chestnut flour, coconut flour and konjac flour have also been researched as alternative flours for cookies. (Xu, J., et al.,2020,Di Cairano, M., et al.,2018). Gluten-free bread typically has a less elastic crumb that hardens more quickly and crumbles easily. These products' flavours fluctuate as well, naturally depending on the substances utilised. To achieve adequate bread volume, crumb softness, and shelf life, technologically and nutritionally functional ingredients like hydrocolloids of cereal and non-cereal origin, fruit or vegetable fibre, flax and chia seeds, psyllium, modified starches, and proteins from many sources must be added in addition to basic gluten-free ingredients like gluten-free flours and starches. (Šmídová, Z., & Rysová, J. 2022)

Due to the high number of people who have been diagnosed with coeliac disease and those who want to change their diet, the market for gluten-free products as well as those aimed at a healthy diet is expanding globally (Stoin, D., et al.,2021). Cookies are one of the most popular and widely consumed food products in India (Shukla and Choudhary.,2022). Due to its appealing qualities including a longer shelf life, a variety of tastes and textures, as well as widespread consumption, cookies are a flexible snack in the food industry and one of the bakery products(Arepally D et al.,2020). gluten-free baked products may have futuristic marketing scope specially for individuals with gluten sensitivity.

Hence the study **"Development of Value-Added Gluten-free cookies for Gluten-Sensitive Disease"**is a humble attempt by the investigator to offer an alternate gluten-free snack for individuals with gluten sensitivity. Thus, the above thesis was carried out with the following objectives.

Primary Objectives:

- Development of a value-added product for gluten-sensitive disease

Secondary Objectives:

- Study the perception on gluten-sensitive disease among dietitians and doctors
- Select and identify food ingredients for gluten-free cookies
- Formulate gluten-free cookies
- Test organoleptic acceptance of formulated gluten-free cookies
- Evaluate the nutrient and nutraceutical properties with special reference to gluten content of developed cookies and
- Analyse the shelf life and product labelling of developed gluten-free cookies

REVIEW OF LITERATURE

II REVIEW OF LITERATURE

The review of literature pertaining to the study on "**Development of Value-Added Gluten-free cookies for Gluten-Sensitive Disease**" is discussed under the following headings:

A. Gluten-Sensitive Disease-An Overview

B. Prevalence Of Gluten-Sensitive Disease-An Eye Opener

C. Causes For Gluten-Sensitive Disease

D. Dietary Management Of Gluten-Sensitive Disease

E. Gluten-Sensitive Disease in Children

F. Novel Food Products For Gluten-Sensitive Disease

G. Indigeneous Medicine For Gluten-Sensitive Disease

A. Gluten-Sensitive Disease-An Overview

In the words of Aljada, B., et al.,2021 Celiac disease is an autoimmune disorder that affects 1% of people and causes reversible inflammation in the small intestinal mucosa in addition to acute symptoms such as diarrhoea, constipation, bloating, nausea, and vomiting. The study also stated that the mucosal injury and inflammation caused due to CD can cause malabsorption of nutrients including calcium, vitamin D, iron, vitamin B12, folic acid, and zinc which when happens over the long term, can have crippling effects like osteoporosis, anaemia, and stunted growth. Lebwohl, B. et al. 2021 described Celiac disease as an immunological-mediated disorder characterised by small intestinal enteropathy, systemic symptoms associated to malabsorption and/or immune activation, and auto-antibodies to tissue transglutaminase (TTG).

The resultant spectrum of CeD presentations is broad, according to Al-Toma, A et al., 2019, and includes extra-intestinal manifestations such as neuropsychiatric disorders, infertility, and liver diseases in addition to gastrointestinal and nutritional derangements brought on by the enteropathy.

According to Singh, A., et al. 2019, celiac disease affects about 1% of the world's population and gluten sensitivity and related illnesses are on the rise globally. According to the authors, up to 13% of people globally are thought to have non-celiac gluten sensitivity, while the prevalence varies depending on the demographic investigated and the diagnostic standards applied. This also covers the difficulties in identifying and treating gluten sensitivity in various groups and geographical areas, including limited knowledge of and access to diagnostic tests, variations in dietary practices, and

cultural beliefs. The authors stress the need for more information, research, and education to advance the identification and treatment of gluten sensitivity worldwide.

B. Prevalence of Gluten-Sensitive Disease- An Eye Opener

Although Non Celiac Wheat Sensitivity (NCWS) is frequently regarded as a very recent clinical condition, it was first described more than 40 years ago by Ellis and Linaker, who described the case of a 43-year-old patient with diarrhoea who did not have celiac disease and who significantly improved after cutting out gluten from the diet.(Sergi, C.,et al.,2021)

Roszkowska, A., et al., 2019 claims the prevalence of Non Celiac Gluten - Sensitivity (NCGS) varies depending on the diagnostic standards employed from 0.5% to 13% of the general population. According to the article, it is more challenging to quantify the prevalence of Non-Celiac Gluten Sensitivity (NCGS) than it is to estimate the prevalence of celiac disease, which is thought to be around 1%.

According to the study conducted by Leonard, M et al, there are 5.6% more people worldwide than normal who have gluten-related illnesses, with celiac disease accounting for 3.1% of these cases and Non-Celiac Gluten Sensitivity (NCGS) for 1.5%.The study also discovered that there were significant regional differences in the prevalence of on-Celiac Gluten Sensitivity (NCGS), with South America having the highest rates (2.6%) and Africa having the lowest rates (0.2%). The study made the case that variations in genetic and environmental factors, as well as differences in diagnostic standards and awareness of the disorder, may be the causes of the prevalence.

Zong, G., & Lebwohl, B. (2020)found that there are significant regional and population differences in the prevalence of gluten sensitivity. The incidence of gluten sensitivity ranged from 0.6% to 6% in the general population and from 3.5% to 15% in patients with functional gastrointestinal disorders. Their studies were according to a review of publications published between 1991 and 2019. According to Aboulaghras, S., et al., 2023, 0.71% of adults in the US are estimated to have celiac disease. According to Stoin, D., et al., (2021), the condition affects more than 1% of the world's population, with lows of 0.5% and highs of 1.26% in North America and Europe. Between 250,000 and 1,000,000 patients in Romania now have celiac disease, up to five to ten times more than there were ten years ago. This is due to both an increase in the incidence of diagnosis and an increase in autoimmune illnesses in general.

A comprehensive assessment of the prevalence of celiac disease conducted by Singh, P. et al. in 2018 revealed a seroprevalence rate of 1.4%, with variations by continent from 1.3% (South America) to 1.8% (Asia).

According to Gupta RS et al., 2019, food allergies affect about 8% of kids in Western countries and are spreading to other parts of the world, particularly urban areas as opposed to rural ones, such as Vietnam, South Africa, and Asia. The prevalence of food allergies in both adults and children is currently estimated to be approximately 10%, indicating that the incidence of food allergies has been rising internationally in recent years. A study by Patel, N., & Samant, H. (2022) suggested that In the US, celiac disease, a non-IgE mediated food allergy to wheat, affects 1% to 2% of the population.

Balakrishnan, V., & Unnikrishnan, A. G. (2015) conducted a study and found a prevalence of non-celiac gluten sensitivity (NCGS) of 6.3% in a group of individuals with functional gastrointestinal diseases in India. They have also concluded that there is a lack of information on the prevalence of NCGS in India, and additional study is required to determine the full scope of this ailment among Indians.

A study on 49 individuals with suspected NCGS was undertaken in 2016 by Ruchika Gupta et al. in India. A double-blind, placebo-controlled gluten challenge and a gluten-free diet were used to evaluate the patients. Based on their symptoms and how they responded to the gluten challenge, the study determined that 12 of the 49 individuals (24.5%) had NCGS. The study also discovered that NCGS patients experienced a wide range of symptoms, such as fatigue, bloating, diarrhoea, and abdominal pain.

Ashtari, S., et al (2019) conducted a study on the prevalence of gluten-related illnesses in Indian patients with irritable bowel syndrome (IBS). The study discovered a greater prevalence of NCGS, which was diagnosed in 6.7% of patients, even though the celiac disease was uncommon in this community, accounting for only 0.6% of patients.

Celiac disease also termed Gluten-sensitive enteropathy, is a chronic autoimmune condition that damages the small intestine in people with a genetic predisposition. With an estimated frequency of 0.3% to 1% among the general population, celiac disease is a developing public health concern in India. A study was carried out by Singh P et al. in 2021.183 IBS patients who had upper gastrointestinal endoscopy and duodenal biopsy was included in the study. Of these, 25 (13.6%) had duodenal biopsy samples that were histopathologically examined and found to have celiac disease. The authors of the study concluded that celiac disease should be taken into account as a potential underlying cause of IBS in people in North India.

In 2019, Makharia, G. K., and Catassi conducted a survey. In total, 43,955 people in Asia had biopsy-proven CD at a frequency of 0.5%. As was to be expected, women had a higher prevalence of CD than men (0.5% vs. 0.4%). In Iran, Turkey, India, and Israel, the combined prevalence of CD was 0.3%, 0.5%, 0.6%, and 0.7%, significantly. In comparison to Iran, Israel and India had a significantly greater overall prevalence of Celiac Disease.

In a study published in 2020, Makharia, G. K., et al. revealed that the prevalence of celiac disease in India was rising and was estimated to be around 1.04%. They discovered that the prevalence of celiac disease ranged between 0.2% and 2.2% in different locations of the study, which was done in five important Indian towns. According to the study, people with a family history of celiac disease and women are more likely to get the ailment. In a study from India in 2020, Balekuduru, A. B., et al. found that only 3 (0.8%) of the 362 IBS patients had biopsy-verified CD.

According to research by Baijal R et al. from the year 2021, celiac disease prevalence has dramatically increased in India over the previous ten years. The prevalence of the celiac disease among patients with chronic diarrhoea grew from 1.79% in 2010 to 5.17% in 2019, according to the study, which was carried out in a tertiary care facility in north India.

In South India, celiac disease was underdiagnosed, with many cases remaining unnoticed, according to research by Kumar P., et al. published in 2020. Only 27% of patients with suspected celiac disease had the diagnosis confirmed, according to a study done in a hospital.According to Rajpoot P et al. 2018, 6.4% of patients with irritable bowel syndrome (IBS) in southern India had celiac disease. The study involved individuals from four states, including Tamil Nadu.

In a study conducted by Bansal D et al in 2019, patients from many Indian states, including Tamil Nadu, participated. They discovered that 9.2% of patients with IBS-D had gluten sensitivity. According to the study, patients with gluten sensitivity were more likely to experience anxiety and despair than patients without the condition.

C. Causes For Gluten-Sensitive Disease

According to Shewry PR, A large amount of wheat is produced, including new varieties with high gluten content, as a result of agricultural mechanisation, genetic modification, and due to usage of industrial pesticides, and nitrogen-based fertiliser. These gluten-rich kinds of wheat are utilised by the world food market. The fast shifts in the amount and kind of wheat ingested may be to blame for the global rise in GRD prevalence. Additionally, using Baker's yeast in place of sourdough prevents the immunodominant gluten peptides from degrading as much. This modification in cooking methods may also contribute to the increased gluten content of wheat in recent years' rising GRD prevalence.

According to research by Caminero, A., et al. from 2019, an immunological reaction to specific gluten proteins may be the origin of gluten sensitivity. Particularly, it has been proposed that a class of proteins called amylase-trypsin inhibitors (ATIs) may operate as a starter for immunological responses in the gut. Wheat and related cereals contain ATIs, which are known to activate a particular class of immune cells known as toll-like receptors and cause inflammation.

A study was done by Mumolo, M. G. et al.202 to identify additional causes of gluten illness. The non-gluten protein fraction includes a class of at least 11 proteins known as wheat -amylase/trypsin inhibitors (ATIs). They make up between 2 to 4% of the total protein content of wheat and are categorised as monomeric, dimeric, and tetrameric forms. The endosperm of wheat seeds contains ATIs, which have multiple functions include regulating starch metabolism during seed development and germination, suppressing enzymes with amylase and trypsin-like activity, and acting as a natural defence against insects and parasites. ATIs were found to be important allergens in baker's asthma as well as innate immune system stimulators. By activating the TLR4-MD2-CD14 in human cells, ATIs enhanced innate immunity.

Although the evidence for these causes is less certain, Mumolo, M. G., et al.,2020 suggested that stress and other environmental variables may be potential causes of gluten sensitivity.As per Koumbi, L., et al. (2020)Short-chain fructose oligosaccharides (fructans), galactooligosaccharides (GOS; stachyose, raffinose), disaccharides (lactose), monosaccharides (fructose), and polyols (sugar alcohols), which are poorly absorbed in the human small intestine and are partially fermented in the large intestine by gut bacteria and yeast and the wheat amylase trypsin inhibitors (ATIs). are all the causes of gluten sensitivity

According to Serena, G., et al. 2019, The development of celiac disease may also be influenced by environmental factors, such as the timing of gluten introduction in infancy and exposure to specific viruses.

In the words of Aboulaghras, S., et al. 2022, the main contributing factor to celiac disease is genetics. Specifically, the part that specific genes play in the body's immunological reaction to gluten, like HLA-DQ2 and HLA-DQ8. Although not everyone with these genes will experience celiac disease, he claims that those who carry them are more likely to be affected.

According to Iqra, Sughra, K., et al (2023), individuals at risk may develop an allergic reaction to wheat proteins like gliadins and glutenins additionally addresses the hereditary component of wheat allergy, pointing out that certain genetic abnormalities may increase a person's risk of getting the disorder. The actual aetiology of celiac disease is unknown, according to a study by Stoin, D., et al. published in 2021. But medical research has recently discovered specific genes that identify a heightened vulnerability to the condition, which is more common in Europe.

The development of wheat allergy may be influenced by hereditary factors, according to Holloway, J. W. (2020). In particular, the FCER1A gene, which produces IgE antibodies that bind to wheat proteins and set off an allergic reaction, the study also makes it clear that in people with weakened immune systems, environmental variables such as being exposed to specific viruses and pollution may raise the likelihood of acquiring a wheat allergy.

Wheat processing, including milling and fermentation, can change the structure of wheat proteins and may make them more allergic, according to Zimmermann, J., et al.(2021).According to a study by Patel, N., & Samant, H. (2022), wheat allergy is a symptom of the release of mediators from mast cells and basophils, including histamine, platelet activator factor, and leukotrienes. The type 2 helper T cell-biased immunological dysregulation that results in sensitization and B-cell IgE production is assumed to be the cause of the IgE production, which is thought to be caused by a rupture of oral tolerance. Wheat flour contains the allergens gliadin, thiol reductase, 1-cys-peroxiredoxin, alpha-amylase/trypsin inhibitor, peroxidase, thioredoxin, lipid-protein transfer, serine proteinase inhibitor, and thaumatin-like protein (TLP).

D. Gluten-Sensitive Disease In Children

According to Sharma P., et al's research from 2020, there were 1.23% of children in India had celiac disease. The study also discovered that the age of onset for celiac disease was commonly between 6 and 10 years and that it affected females more frequently than boys. A study by Rajpoot P et al(2018) employing individuals from four states, including Tamil Nadu, discovered that women were more likely than men to have celiac disease. According to Collin, P., et al. (2018), CD is diagnosed more frequently in females with an average Female:Male ratio of 2:1 and an onset following a bimodal age distribution, with an initial peak in the first two years of life and a second peak in the second or third decade, even though about 25% of all diagnoses happen at an age of 60 or older.

According to Popoviciu, M. S., et al. 2023, women and those with specific autoimmune illnesses, like Hashimoto's thyroiditis or type 1 diabetes, may be more likely to have gluten sensitivity. Cabanillas, B. (2020) stated that gluten sensitivity is more frequently diagnosed in adults between the ages of 30 and 50 and Szakos, D., et al. (2020) stated that gluten sensitivity may be more common in older persons, particularly those over the age of 60. The research of Caio, G., et al. (2019) stated that Celiac disease may develop at any age, from infancy to old age. according to his study, there are two peaks in onset: one arises shortly after weaning off of gluten in the first two years of life, and the other occurs in the second or third decades. A study by Patel, N., & Samant, H. (2022) suggested that Children are more prone to develop a wheat allergy than adults are, and they are more likely to do so if wheat is introduced to them after they are six months old. Most allergic children outgrow their condition, and by the age of 12, roughly 65% of cases are resolved.

E. Dietary Management For Gluten-Sensitive Disease

DRUG THERAPY

Aspergillus niger-Prolyl Endopeptidase (AN-PEP) is a glutenase with peak activity between 3 and 5 in the gastric pH. Therefore, the potential for AN-PEP as an oral supplement has been investigated. Even in complicated food matrices like fast food meals, the enzyme has been shown in preliminary investigations to catalyse the virtually full destruction of gluten epitopes. Additionally, when AN-PEP and gluten were given together to CeD patients, T-cell stimulatory peptides from both gliadins and glutenins were eliminated within 2 hours as seen in the stomach aspirates of the patients.(Scherf, K. A.,et al.,2018)

Aljada, B et al 2021 conducted research and found that 95% of children can have their small intestinal architecture restored with a rigorous GFD in just two years, compared to 34% and 66% of adult patients who exhibit mucosal recovery after two and five years, respectively. However, some studies indicate that older patients (between the ages of 30 and 60) only partially recover, while people over the age of 60 do not statistically recover. A GFD can help with malabsorption symptoms such as diarrhoea, steatorrhea, and weight loss as well as small bowel healing. A complete reversal of osteopenia could not be seen, although some studies have shown a considerable increase in bone mineral density after one year of the diet.

Sublingual specific immunotherapy (SLIT) was used to treat workers with asthma and/or rhinitis brought on by wheat flour in a study by Dubini, M., et al., published in 2020.In the bakery environment, there are a number of potential sensitizers, but wheat flour seems to be the main one. Five bakers chose to receive SLIT because medication therapy and preventative measures were ineffective. Sublingual wheat flour extract administration was used to guide a three-year investigation. Prior to and following SLIT, questionnaires, allergy and pulmonary testing were conducted.Every patient experiences symptom relief following SLIT; improved scores on the Asthma Control Test and a quality-of-life

Oral immunotherapy (OIT) was used in a trial on the management of severe food allergies undertaken by Babaie, D., et al.2022 The study's participants are children aged 2 and older with a history of wheat allergy. A double-blind, placebo-controlled meal challenge proved that the patient had a wheat allergy.

Oral immunotherapy (OIT was initiated in order to supply 5.28 g of wheat protein in 60 g of bread. In addition to immunologic tests, a second and third oral food challenge (OFC) were conducted to assess the long-term effectiveness of wheat OIT (WOIT) after 3 months and a year of maintenance therapy.Eight of the seventeen patients who completed the three-month maintenance phase had

negative OFCs. All nine of the nine with positive OFCs were instructed to continue consuming 60 g of bread each day for an additional 12 months.The findings suggested that, when used under close supervision, wheat oral immunotherapy (WOIT) is a safe and efficient form of treatment.

To assess therapies for CeD and refractory CeD, numerous clinical trials are now being conducted. A study on nondietary therapy was done by Kulkarni, A., et al. in 2021 with the goal of assisting CeD patients who follow a gluten-free diet in preventing and protecting themselves from acquiring major secondary disorders upon accidental gluten ingestion.Larazotide acetate, derived from the zona occludens toxin of Vibrio cholera, is a first-in-class tight junction regulator for CeD.Larazotide acetate has been proven in preclinical trials to increase tight junction adhesion, macrophage activity, and improve small intestine permeability, all of which inhibit the inflammatory response brought on by consuming gluten

A gluten-specific protease called latiglutenase (IMGX-003, originally ALV003) is now being studied as a CeD therapy. Orally given, it combines prolyl endopeptidase (PEP) and cysteine protease (EP-B2). In vitro tests have revealed that both proteases can break down gluten. Upon administration, latiglutenase prevents gluten from passing through the mucosal barrier by securing the tight junctions in the small intestine. Latiglutenase may be helpful in easing the symptoms of CeD, but it cannot repair the epithelial lining that has already been injured by consuming gluten in the past.

The medication Kuma030 from PvP Biologic is a gliadin peptidase that breaks down gliadin peptides. According to studies, treating gliadin with Kuma030 rendered it incapable of triggering a T-cell immune response. Additionally, Kuma030 was discovered to be capable of destroying >99% of immune gliadin under laboratory-simulated stomach circumstances, at a level that was safe for CeD patients.Previously known as poly(HEMA-co-SS), BioLineRx BL-7010 binds gliadin intraluminally and stops the production of gliadin-derived peptides in the small intestine. The medication has a significant propensity for binding with gliadin, according to investigations on its in vitro toxicity.

DIET THERAPY

The only available treatment for CD, according to Drabiska, N., et al., 2019, is rigorous adherence to a gluten-free diet (GFD). In CD patients, the symptoms are reversed, the intestinal mucosa is restored, and nutrient absorption returns to normal when gluten is completely cut out of the daily diet. Additionally, most CD patients with this diet have higher levels of bone mineralization. However, compared to the healthy population, some studies found that CD patients' BMD was lower after consuming a GFD. This could result from a diet that is out of balance or from inadequate GFD compliance. GFD use alone may lead to other deficiencies, such as those in calcium phosphate and vitamin D, which could have a detrimental effect on bone health.

Martnez-Villaluenga, C., et al. 2020 stated that due to its outstanding nutritional and nutraceutical value and status as gluten-free (GF) grains, pseudocereals are currently popular in human diets. Saponins, phenolic compounds, phytosterols, phytoecdysteroids, polysaccharides, betalains, and bioactive proteins and peptides are only a few of the classes of bioactive substances found in pseudocereal grains. The consumption of pseudocereals or their bioactive components has been linked to benefits against obesity, pre-diabetes, and problems from diabetes. The study concluded that the lack of gluten in these pseudocereals has made them an excellent alternative for the development of tasty GF goods that enable a sufficient intake of nutrients in persons with celiac disease, the number of whom is increasing daily.

Pearl millet has a better nutritional value than other major grains, according to research by Pei, J., et al., published in 2022. This is because it contains a lot of proteins, minerals, phytochemicals, vitamins, and dietary fibre.Benefits of pearl millet include increased haemoglobin, anti-allergic, constipation relief, anti-ulcerative, weight loss, required for bone growth and development, protection from cardiovascular diseases, management of blood pressure, and assistance with celiac disease.

As per Sood, S., and Mohiuddin Bhat, F., 2021 Ragi, often called finger millet, is a grain that doesn't contain gluten and has a low glycemic index, making it a nutraceutical. Finger millet is an excellent source of calcium, phosphorus, magnesium, and iron in addition to the vitamin B complex, which includes thiamine, riboflavin, folic acid, and niacin. Additionally, finger millet is beneficial for those with diabetes mellitus and digestive disorders due to its low fat content. According to Rana, S., & Bhandari, N. S. 2023, finger millet contains polyphenols, pigments, phytates, and amino acids including lysine, valine, tryptophan, methionine, threonine, leucine, and isoleucine.

According to Vanniarajan, C., et al.,2020, banyard millet is a good source of natural antioxidants since it contains phytochemicals such phenolic acid, flavonoids, and tannins.Vanga, S. K., et al. 2018 state that barnyard millet contains a list of amino acids that includes valine, isoleucine, leucine, lysine, phenylalanine, histidine, threonine, tryptophan, methionine

Using an ELISA test, Yu, J. M., et al. (2021) conducted a study on the gluten content of several food products. A storage protein known as gluten is present in wheat, rye, barley, and their hybrids. Gluten can be separated into prolamin groups that are alcohol-soluble and glutelin groups that are acid-/alkali-soluble based on their solubility. Wheat contains the glutelin group's glutenin. Wheat, barley, rye, and oats all include the prolamins gliadin, hordein, secalin, and avenin. Prolamin is typically estimated to make up about 50% of gluten. Some people's intestinal mucous membranes may severely shrink as a result of this dietary gluten, which will prevent nutrients from being absorbed and result in Gluten-related disorders.

According to the Food and Drug Administration of the United States (FR Doc. 2013-18813) Products labelled as "food specifically processed to reduce gluten content" and "low gluten-level" must adhere to gluten content standards of 20 to 100 mg/kg. For this work, the researchers used gliadin from wheat and secalin from rye were tested using ELISA techniques with R5 and G12 antibodies. alcoholic beverages, bread, noodles, powdered foods, and snacks were used for analysed. In a blender, each sample was homogenised. Quantitative analysis was conducted using the RIDASCREEN Gliadin test kit, the Veratox for Gliadin R5 test kit, and the AgraQuant Gluten G12 test kit. The findings showed that gliadin quantitation values from the Veratox for the gliadin R5 kit and the RIDASCREEN gliadin assay were twice as high as the estimated gluten concentration.

F. Novel Baked Food Products For Gluten-Sensitive Disease

Woomer, J. S., and Adedeji, A. A. (2021) conducted a study on gluten-free grains and formulated bread and snack products from Gluten free ingredients Sales of GF products were 4.63 billion USD in 2017 and are anticipated to expand by 7.6% annually to 6.47 billion USD by 2023. A gluten-free diet mostly consists of grains like millet, corn, sorghum, and pseudocereals like amaranth, quinoa, and teff. Customers are demanding healthier food options as they become more conscious of the impact that nutrition plays on their health and well-being. Additionally, improved gastroenterological problem diagnosis is fueling the food trend. At the beginning of the century, the market for GF foods was only available in specialised and health-food supermarket stores. However, the demand for GF products has increased during the past 20 years.

The consumption of GF foods and beverages is increasing, and this trend is anticipated to last into the future. The elastic properties of gluten are well known for causing the bread to rise and create air bubbles, which results in a porous structure. Although there aren't many raw foods that contain gluten, GF users still need to steer clear of a lot more goods that use gluten as a functional ingredient. Many commonly consumed items, including bread, pasta, snacks, and beverages, are now made using GF grains and crops, such as corn, rice, millet, amaranth, teff, cassava, tapioca, and quinoa. Because gluten-free foods lack the viscoelastic quality that gluten imparts, product manufacturers have faced difficulties while creating GF products. The functional elements used to enhance the quality of bread, including hydrocolloids, enzymes, proteins, and starch, are a major emphasis of GF goods.

Pseudocereals (quinoa, amaranth, buckwheat), cereals (millet, sorghum, teff, maize, rice), legumes (chickpea, soy, carob germ), and pseudocereals (sorghum, teff, buckwheat) are among the alternative bread flours being explored. For successfully enhancing the physical characteristics of GF bread, both plant-based and animal-based proteins have been employed. The inclusion of proteins (albumin, collagen, pea, lupine, and soy) had a favourable impact on nutritional value whereas rice bran protein concentrate was observed to boost flexibility, gas retention, and shelf-life of GF bread. Dairy-

derived proteins have the potential to enhance the properties of GF dough. Whey protein isolate (WPI) demonstrated the greatest improvement in bread quality, while sodium caseinate (SC) and milk protein isolate (MPI) has been proven to enhance the qualities of gluten-free batter flexibility.

According to the presence of CD, Tristan Asensi, M., et al. (2023) conducted a study to determine the consumption of ultra-processed foods and its link with Mediterranean Diet adherence in a group of individuals. Ultra-processed foods made up 14.5% of the diet of individuals with CD (246 g/day), with sweets and cereal-based goods accounting for the majority of the remaining 25.2%.

Consumption of UPF was unaffected by the presence of CD, while persons with CD consumed precooked pasta and prepackaged bread at considerably greater rates. The subjects included were 312 adults without CD and 103 adults with CD. Through the use of a self-administered questionnaire, data were gathered. The result indicated that the amount of UPF being consumed did not change when CD was present. The most common UPF in the diets of persons with CD were cereal-based products and sweets. However, subjects with CD admitted to consuming more precooked pasta and prepackaged bread than subjects without CD. On the other hand, those with CD consumed more non-traditional Mediterranean foods and had much lower MD adherence than participants without CD.

In 2019, Torbica, A., et al. created a unique bread employing non-wheat flour. The goal of the study was to combine thermal and hydrothermal pretreatments of flour to create additive-free, gluten-free loaves. By using the standard breadmaking technique, new rye, oat, sorghum, and millet bread based on a blend of comparable heat-treated and extruded flours in a ratio of 70:30 were created. In this method, there were no additives applied. The loaves that were produced have more fibre than usual. All bread resembled wheat bread in appearance. Gluten-free flours resulted in harder, less elastic, and more granular bread.

Arribas, C., et al.,2019 saud that using a variety of gluten-free formulations made from a combination of the carob fruit, pea, and rice flour, the impact of extrusion on the number of bioactive substances (inositol phosphates, -galactosides, protease inhibitors, lectins, and phenolic compounds) were found. Results were compared with two commercial snacks as well as their non-extruded formulation counterparts

A gluten-free cake was developed in 2022 by Levent.H et al.The gluten-free flour mixture made up of rice flour (75%), chickpea flour (15%), and carrot flour (10%) was used to make the cake. To boost the nutritional value of gluten-free cakes, this mixture was replaced separately with 5% each of grape seed, pomegranate seed, flaxseed, poppy seed, and turmeric. In comparison to the gluten-free control sample, the supplemented cakes had greater values for ash, crude protein, total phenolic content, and antioxidant activity. Pomegranate seed and flaxseed cake samples had higher calcium, Phosphorus, and potassium levels than control cakes. Additionally, cakes containing flaxseed and poppy seeds were discovered to be richer in Manganese and Magnesium than the control. The results showed that grape

molasses combined with flaxseed and poppy seeds might be advised for the creation of gluten-free cakes with acceptable sensory qualities, high total phenolic content, and antioxidant activity.

G. Indigenous Medicine For Gluten-Sensitive Disease

Nayak, S. N., et al.,2021 investigated the potential of Terminalia chebula, commonly known as "Haritaki," in managing gluten-related disorders. Haritaki is a medicinal plant that has been used in Ayurveda, the traditional Indian system of medicine, for various health issues, including digestive disorders. The study found that Haritaki can reduce inflammation in the gut caused by gluten. Haritaki was shown to inhibit the release of pro-inflammatory cytokines and increase the production of anti-inflammatory cytokines in intestinal cells exposed to gluten. Additionally, Haritaki was found to protect the intestinal barrier by increasing the expression of tight junction proteins, which help maintain the integrity of the gut lining.

A systematic review and meta-analysis were conducted in 2020 by Seiler, C. L., et al. to assess the effectiveness of probiotics in reducing GI symptoms and raising patients with CD's quality of life (QOL). According to the GI Symptoms Rating Scale, probiotics reduced GI symptoms.After taking probiotics, Bifidobacteria levels rose.According to the study, probiotics may help CD patients with their GI issues.

The key ingredient of turmeric, curcumin, was the subject of a 2021 study by Gupta, K. B., et al. They discovered that curcumin can lessen inflammation in the gut brought on by gluten. In intestinal cells exposed to gluten, the study found that curcumin inhibited the release of pro-inflammatory cytokines, which are proteins that cause inflammation.

Asri, N., et al 2021 said that In order to prevent or treat inflammatory illnesses, herbal remedies and other natural resources have demonstrated anti-inflammatory effectiveness.Plant secondary metabolites known as polyphenols have the potential to significantly improve human health. Antioxidant and anti-inflammatory properties are present in polyphenols.Arotenoids are tetraterpenes, which are dietary fat-soluble substances that are taken up by intestinal cells. Adopting carotenoids can be thought of as a beneficial dietary therapy for celiac disease since they play a part in reducing oxidative stress, keeping intestinal barrier integrity, and regulating the risk of intestinal chronic inflammatory diseases.According to their Cinnamon, cayenne, Echinacea, cocoa, green tea, black seeds and cloves, can also help to manage inflammatory diseases.

METHODOLOGY

III METHODOLOGY

The study entitled **"Development of Value-Added Gluten-Free Cookie for Gluten-Sensitive Disease"** was carried out in two phases and is discussed underneath and illustrated

PHASE -I

Perception of gluten-sensitive disease among dietitians and doctors in Coimbatore

Selection of area

Selection of subjects

Collection of data and conduct of the study

Software used for analysis

Analysis of data

PHASE -II

Formulation of gluten-free cookies

Selection of Ingredients

Processing of ingredients

Formulation of recipes

Method of preparation

Analysis of product quality

Labelling and packaging

PHASE -I

Perception of gluten-sensitive disease among dietitians and doctors in Coimbatore

Selection of area

Since the investigator hails from the city of Coimbatore, the area of Coimbatore was selected.In Coimbatore, three popular multispeciality hospitals with well-established dietary departments were selected for the conduct of the study based on convenience sampling. The investigator obtained the consent for collecting data from institutions and the study was placed for ethical clearance under the Institutional Human Ethics Committee

Clearance number: IHEC/22-23/FSMD-01.

Selection of subject

A total of 30 respondents comprising 17 Dietitians and 13 Doctors working in selected three multispeciality hospitals were purposively selected to study the perception of the gluten-sensitive disease. The term purposive sampling describes a range of non-probability sampling procedures where units are chosen because they have the qualities that investigator need sample(Alvi,M.,2016).Therefore, in purposive sampling, units are chosen "on purpose".Inform consent from dietitians and doctors was obtained for the participation in the study

(PHASE-I)

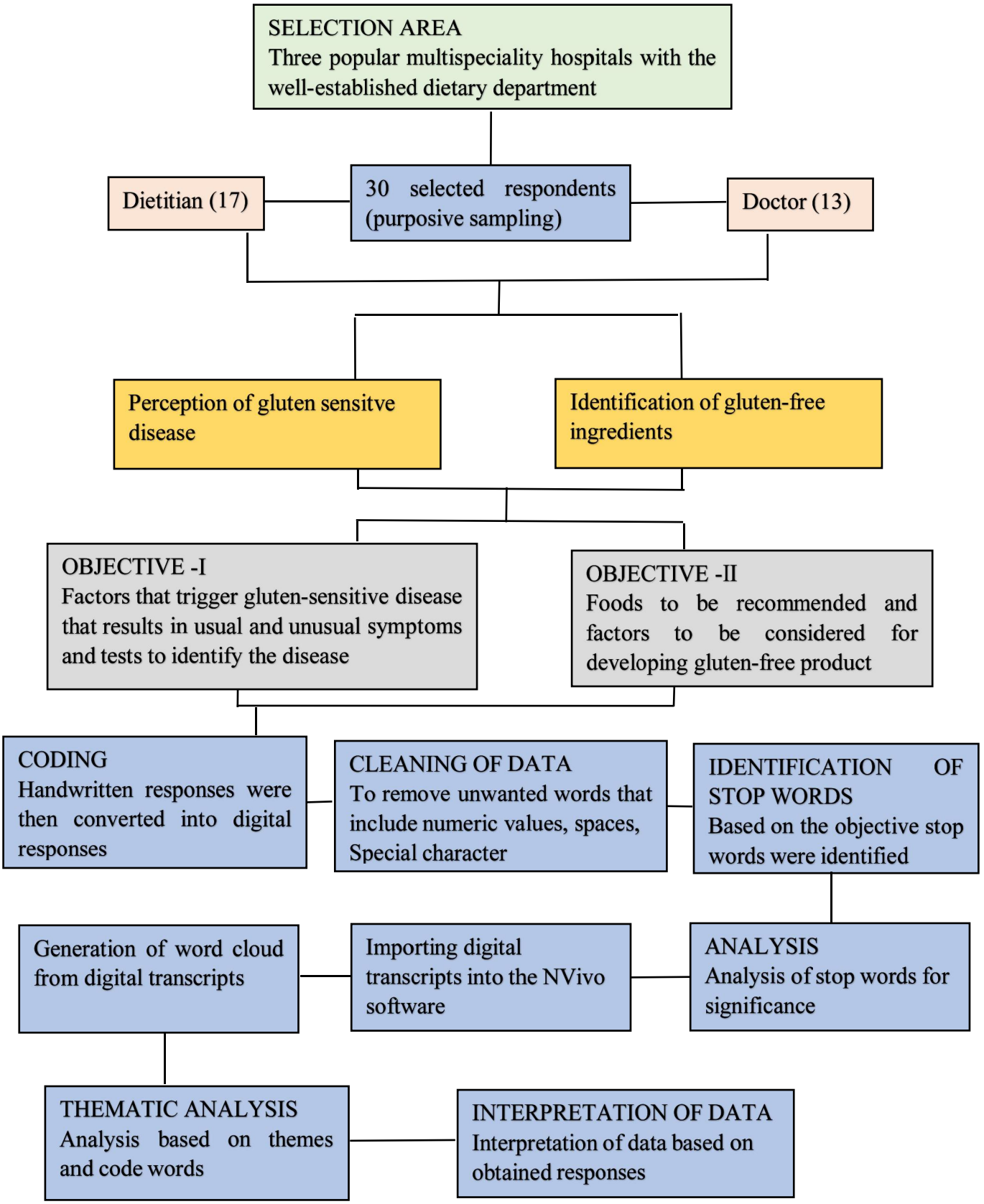


Figure 1

Data Collection and Analysis

Collection of data and conduct of the study

To test the perception of gluten-sensitive disease from dietitians and doctors and the selection and identification of ingredients in foods to be recommended for gluten-sensitive disease, a well-structured open-ended questionnaire comprising nine questions was formulated by the investigator. The developed questionnaire was pretested among five respondents to validate the clarity of the question asked, concepts and contextual meaning of the question. The formulated questionnaire was then administered to the selected 30 dietitians and doctors

Software used for analysis

NVIVO is a software program used for qualitative and mixed-methods research. It is specifically used to analyse unstructured text, audio, video, and image data from sources such as interviews, focus groups, surveys, social media, and journal articles. It is a leading qualitative analysis solution. To eliminate the effort of hand transcriptions, NVivo Transcription is smoothly linked with NVivo 14. By using automated transcriptions from high-quality audio and video formats, the transcription add-on enables researchers to skip the hard transcription procedure with 90% accuracy. With the synchronised audio playing, a native editor makes changes faster, tags speakers, and ensures proper formatting. Thus the NVivo software was used to analyse the response of the dietitians and doctors

Analysis of data

As part of the analysis, the investigator coded the text by compiling the answers from dietitians and doctors into a single response sheet, for every question asked. The collected responses were converted into digital responses and the data was cleaned by removing unwanted words such as numerical values, and first-person usage (I, We, Me). Uniformity of cases was done for all the questions. Finally, stop words were defined by investigators (Eg- foods, symptoms, tests etc) and transcripts were imported into NVivo software for analysis. As a result of the analytical output, a word cloud was created, and the major content of the analysis was extracted from it before thematic analysis using the auto code feature. The data gathered from the interview transcripts was combined with text mining and qualitative content analysis was done to show thematic convergence.

A research technique called content analysis is used to find specific words, themes, or concepts in a given set of qualitative data. They are used to quantify and examine the occurrence, significance, and connections of such specific words, themes, or concepts using content analysis.

Thematic analysis is a technique for analysing qualitative data in which a set of data is read through and themes are discovered by looking for patterns in the meaning of the data.Qualitative research frequently uses thematic analysis. It places a focus on spotting, investigating, and deciphering qualitative data patterns.

PHASE -II

Formulation of gluten-free cookies

Selection of ingredients

The prevalence of food allergies in both adults and children is now estimated to be around 10%, suggesting that the incidence of food allergies has been rising globally in recent years. (Gupta RS et al., 2019). According to Sergi., et al.,2021 Roughly 3% of people worldwide experience wheat intolerance (1% with Wheat Allergy,1% with celiac disease, and 1% with non-celiac gluten sensitivity) which lead to deficiency of iron, vitamin D, calcium, vitamin B12, folic acid, and zinc (Kreutz, J. M., et al.,2022). Hence the investigator was motivated to develop a gluten-free cookie.

Wheat, rye and barley are the cereals with gluten protein in them. Wheat contains 75% gluten. Compared to wheat and barley rye tends to have less gluten in it. Except for this other cereals and millets such as rice, maize, and sorghum, pseudocereals such as quinoa, amaranth, and buckwheat and fruits, vegetables and milk will are gluten-free .Recent scientific literature also claims waterchestnut (fruit) and Quinoa (pseudocereal) to be gluten-free. They have nutraceutical properties such as carotenoids, flavanoids and phenols . Since much of the studies were not done using waterchestnut flour and quinoa flour, they were selected as the main ingredients for development of gluten-free cookie . Along with waterchestnut flour and quinoa flour, functional ingredients such as sugar, baking soda, butter, soy lecithin powder and xanthan gum powder were also selected to develop the gluten-free cookie

Processing of ingredient

The identified ingredient such as white sugar ,brown sugar, butter and baking soda was procured from local markets in Coimbatore .Ingredients like waterchestnut flour ,quinoa flour, xanthan gum powder and soy lecithin powder were purchased from mumbai, maharashtra. The ingredients were then stored in air-tight containers and maintained at the required room temperature of 25º celcius.

Formulation of recipes

Seven variations of gluten-free cookies as given in Table 1 were formulated adopting the standard recipe suggested by Thangam E.Philip, Edition -5.

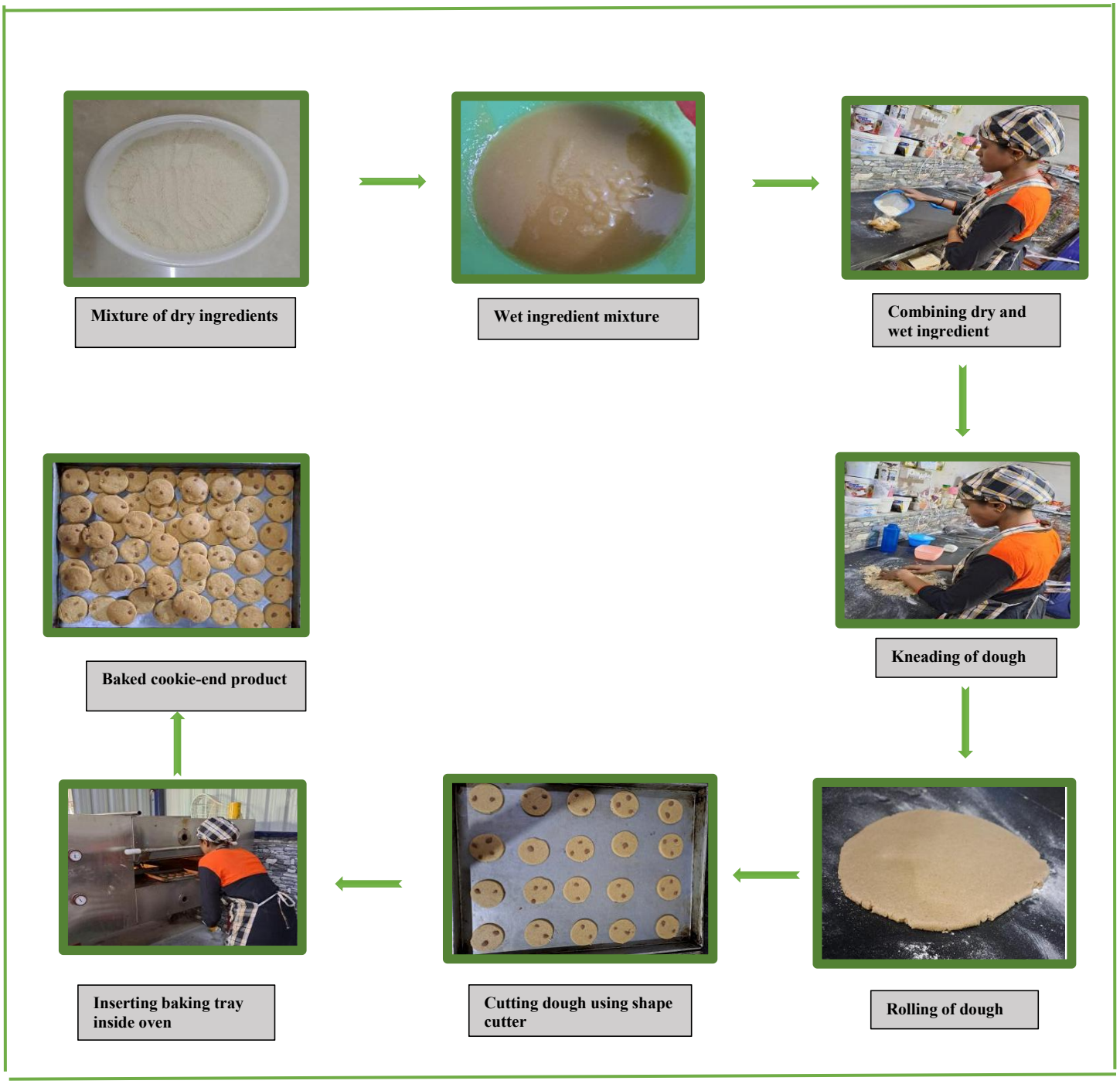
Table-I

Formulation of gluten-free cookies

VARIATION	COMPOSITION
Variation I (W1) (W: Q = 100:00)	170 grams of waterchestnut flour+85 gram butter+60 gram white sugar powder+60 gram brown sugar powder
Variation II (W2) (W: Q = 80:20)	136 grams of waterchestnut flour+34 grams of quinoa flour+85 gram butter+60 gram white sugar powder+60 gram brown sugar powder
Variation-III (W3) (W: Q = 60:40)	102 grams of waterchestnut flour+68 grams of quinoa flour+85 gram butter+65 gram white sugar powder+65 gram brown sugar powder
Variation-IV (WQ) (W: Q = 50:50)	85 grams of waterchestnut flour+85 grams of quinoa flour+85 gram butter+65 gram white sugar powder+65 gram brown sugar powder
Variation V (Q1) (W: Q = 40:60)	102 grams of quinoa flour+68 grams of waterchestnut flour+85 gram butter+60 gram white sugar powder+60 gram brown sugar powder
Variation-VI (Q2) (W: Q = 20:80)	136 grams of quinoa flour+34 grams of waterchestnut flour+85 gram butter+60 gram white sugar powder+60 gram brown sugar powder
Variation-VII (Q3) (W: Q = 00:100)	170 grams of quinoa flour+85 gram butter+60 gram white sugar powder+60 gram brown sugar powder

Method of preparation

A commercial bakery unit was identified by the investigator. The formulated ingredient was combined and kneaded into a dough then cut into flat oval shape cookies of 2 inch diameter and and 0.25 inch widhness using a cookie cutter. After a proofing time of 15 minutes in room temperature the cookies were baked in a manual gas baking oven of 100-500 kg capacity with voltage of 220 v, frequency of 50Hz and power consumption of 2.4 kW/hr. The oven was preheated at 180º celcius for about 30 minutes. The cookie tray was placed in the centre of the oven was baked at 180º celcius for 15 minutes . The baked product was then cooled for 20 minutes to bring back to room temperature.



Method of preparation
Figure-2

Analysis of product quality

Organoleptic analysis

The formulated cookie's were tested for its product quality namely organoleptic acceptability, texture analysis, shelf life and color card score using 20 semi-trained panel member.For sensory evaluation, each semi-trained panel member was given two samples, namely the formulated cookies and a standard cookie. Unibic chocolate chip cookie was selected as standard cookies. The semi-trained panel members were asked to rate the formulated cookie's for its appearance, taste, texture,colour and flavour using a nine-point hedonic rating scale with a maximum acceptability score of 35-45 and a minimum score of 0-25. Based on the cumulative score the acceptability of formulated gluten-free cookies was categorized as not acceptable (0-25),acceptable (25-35) and highly acceptable (35-45) .

Organaloptic analysis

Figure-3

Preference test

Further to identify the most preferred cookie , a preference ranking test was carried out by the investigator, for which the panel members were asked to taste all the seven variations of cookie and to rank them in the order of preference in terms of Appearance, taste, texture, colour and flavour.

Prefernce test

Figure-4

Colour analysis

The surface colour of the formulated product was analysed using a colour spectrometer of light energy across 420-620 nm wavelengths. Colour values of both the standard and the formulated cookie's was analysed on day one by the investigator and the results were compared .Obtained L*,a*,b* indicates L (+ lightness,− darkness), a (+ redness, − greenness), and b (+ yellowness, − blueness) .L represents darkness to lightness, with values ranging from 0 to 100; a represents greenness to redness with values of -128 to +127; and b represents blueness to yellowness also with values from -128 to +127.

Surface colour analysis of gluten-free cookies

Figure-5

Texture analysis

The texture quality of the formulated gluten-free cookies was then analysed using the texture analyser on day one, seven, fourteen and twenty-one days . Using a three-point bending jig with a 100 N load cell capacity, the texture analyzer was calibrated. The calibrating process involved applying force with a 5mm/second stroke rate. Before conducting the texture analysis of the test product, the stroke force was first calibrated at 0. The obtained results were captured in data as graphs.

Shelf-life analysis

Shelf life analysis of the formulated gluten-free cookies was conducted by the investigator for 21 days. The formulated cookies were stored in an airtight container and kept away from direct sunlight. Visual appeal, taste, texture, colour and flavour of the product was observed every day and the product

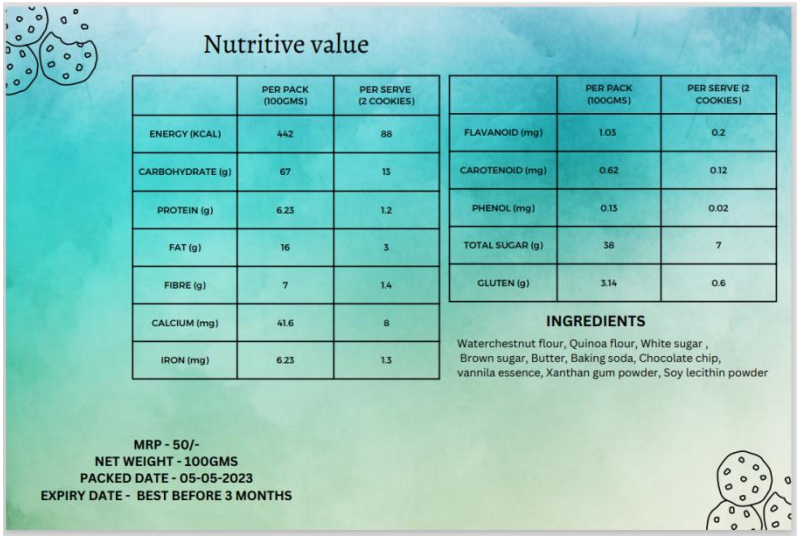
was tested for appearance (visual appeal), texture (moisture), taste (after mouth feels), flavour (rancidity), colour (light exposure) and overall acceptability.

Nutrient Analysis

The cookies were analysed for macronutrients and micronutrients adopting standard procedure namely for Energy (EFLT/SOP/2019), Carbohydrates (IS 1656:2007 RA 2019), Total protein (IS 7219:1973 RA 2020), Fats (IS 12711:1989 RA 2015), Crude fibre (AOAC 20th Edn 944.13), Calcium as Ca (IS 5949), Iron as Fe (IS 12711:1989 RA 2015), Total sugars (FSSAI MANUAL FOR ADDITIVES) Moisture (IS 12711:1989 RA 2015), Ash (IS 12711:1989 RA 2015) and neutraceuticals such as flavonoids (FSSAI MANUAL FOR ADDITIVES), Carotenoid (FSSAI MANUAL FOR ADDITIVES), Phenol (FSSAI MANUAL FOR ADDITIVES). The gluten content of the cookies was also analysed (IS 1155).

Labelling and packaging

Selected variation was then packed per 100 g that comprises of 10 piece of gluten-free cookies.Nutritive value per pack and per serving comprising two cookies was calculated and labelled .The product is packed in 150 ml plastic cookie jar of 10 cm length and 3 cm widthness.The label is shown in below figure



Nutritive labelling of gluten-free cookie

Figure-6

RESULT AND DISCUSSION

IV RESULT AND DISCUSSION

The results and discussion of the present study titled, "**Development of Value-Added Gluten-Free Cookie for Gluten-Sensitive Disease**", are discussed under the following headings:

Collection of data

PHASE -I

Perception on gluten sensitive disease among dietitians and doctors in Coimbatore.

PHASE -II

Formulation of gluten-free cookies

PHASE -I: Perception on gluten sensitive disease among dietitians and doctors in Coimbatore.

Using a well-structured open-ended questionnaire comprising nine questions the perception on gluten sensitive disease among dietitians and doctors from three different multispeciality hospitals was collected by the investigator. The collected data were processed using NVivo software. The response of dietitians and doctors was subjected to content and thematic analysis

The analysis of the response collected from selected subjects revealed that the collected information was divided into two sections based on the objective of the study- Factors that trigger gluten-sensitive disease that results in usual and unusual symptoms and tests to identify gluten-sensitive disease. The responses obtained from dietitians and doctors were then compared .

Symptoms associated with gluten-sensitive disease

Perception of dietitians on symptoms associated with gluten-sensitive disease in the form of the most frequent word is depicted in table-II

Table-II

Perception of Dietitians on Symptoms of gluten-sensitive Disease

Word	Length	Count	Weighted Percentage (%)
Diarrhoea	9	15	5.23
Bloating	8	12	4.18
Abdominal pain	9	10	3.48
Fatigue	7	9	3.14
Headache	8	8	2.79
Nausea	6	8	2.79
Vomiting	8	6	2.09
Diabetes	8	5	1.74
Skin disease	7	5	1.74
Skin rash	4	5	1.74
Stress	6	4	1.39
Anxiety	7	3	1.05
Depression	10	3	1.05
Ulcer	5	3	1.05
Migraine	7	1	0.35
Short Stature	7	1	0.35
Loose stool	5	1	0.35
Anaemia	6	2	0.70
Cramps	6	1	0.35
Dermatitis	10	1	0.35
Brain fog	3	1	0.35

From Table-II it is evident that the dietitian perceived diarrhoea, bloating and abdominal pain as major symptoms of gluten-sensitive disease with a weighted percentage of 5.23%,4.18% and 3.48% respectively.

Perception of doctors on symptoms associated with gluten-sensitive disease in the form of the most frequent word is depicted in table-III.

Table-III

Perception of Doctors on Symptoms of a gluten-sensitive Disease

Word	Length	Count	Weighted Percentage (%)
Constipation	12	13	7.30
Skin Allergy	7	6	3.37
Diarrhoea	9	11	6.18
Skin disease	4	11	6.18
Bloating	8	8	4.49
Headache	8	6	3.37
Anxiety	7	5	2.81
Abdominal pain	9	4	2.25
Foggy brain	5	4	2.25
Skin rash	4	4	2.25
Weight gain	6	4	2.25
Depression	10	2	1.12
Fatigue	7	2	1.12
Mental problem	6	2	1.12
Anaemia	6	1	0.56
Emotional loss	9	1	0.56
Stress	6	1	0.56

From the table-III it is clear that the doctors perceived constipation as the most common symptom(7.30%) followed by diarrhoea (6.18%) and skin disease (6.18%). However, both doctors and dietitians (table-II) perceived bloating as a symptom of gluten-sensitive disease with a weightage of 4%.

Perception on test to identify the gluten-sensitive disease

Perception of dietitians on tests for the gluten-sensitive disease in the form of the most frequent word is depicted in table-IV

Table-IV

Perception of dietitians on test for gluten-sensitive disease

Word	Length	Count	Weighted Percentage (%)
Blood test	4	10	3.48
Antigen test	7	5	1.74
Biopsy	6	5	1.74
Antibody test	8	4	1.39
Endoscopy	9	3	1.05
IgA test	3	3	1.05
Elisa test	5	1	0.35
Itg test	3	1	0.35

From table-IV it is evident that the dietitians perceived blood tests as the most common test for identifying gluten-sensitive disease at a weightage of 3.48% followed by antigen test (1.74%) and biopsy (1.74%).

Perception of doctors on tests for the gluten-sensitive disease in the form of the most frequent word is depicted in table-V.

Table-V

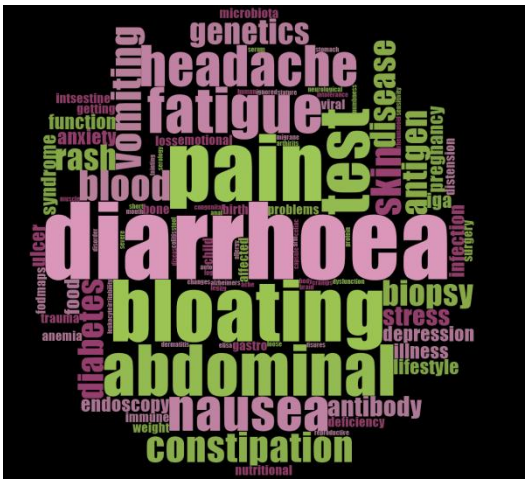
Perception of doctors on test for gluten-sensitive disease

Word	Length	Count	Weighted Percentage (%)
IgA test	3	6	3.37
Immunoglobulin test	14	1	0.56
Serological test	11	1	0.56
Antibody test	8	1	0.56

From table-V it is clear that the doctors perceived the IgA test (3.37%) as the frequently preferred test followed by the Immunoglobulin test (0.56%), serological test (0.56%) and antibody test

(0.56%). However, both dietitians and doctors recommend an Antibody test and IgA test for identifying gluten-sensitive disease.

Content analysis for the perception of dietitians and doctors on symptoms and tests for gluten-sensitive diseases are given below:



Word cloud for the perception of dietitians on symptoms and test for the gluten-sensitive disease

Figure-7

Word cloud for the perception of doctors on symptoms and test for the gluten-sensitive disease

Figure-8

The word cloud in figure-7 to analyse perception of dietitians on symptoms and test to identify gluten-sensitive disease depicts the word "diarrhoea" was highlighted by most of the dietitians as the symptom of gluten-sensitive disease followed by bloating, abdominal pain, constipation, diarrhoea , headache and fatigue . For test to identify gluten-sensitive disease "Antigen test " was the mostly highlighted word followed by endoscopy, antibody test and blood test .

The word cloud in figure-8 to analyse perception of doctors on symptoms and test to identify gluten-sensitive disease depicts the word "constipation" was highlighted by most of the dietitians as the symptom of gluten-sensitive disease followed by bloating, skin allergy , abdominal pain and headache. To identify the gluten-sensitive disease "blood test" was recommended most followed by IgA test .With the help of figure-1,2 , thematic analysis was carried out which helped in forming theme .

Thematic analysis of the perception of dietitians on symptoms to identify gluten-sensitive disease is represented in the below table

Table- VI

Thematic representation of perception of dietitians on symptoms to identify gluten-sensitive disease

Word	No of times repeated
Depression	2
Diabetes	2
Diarrhoea	3
Skin allergy	3
Fatigue	2
Headache	2
Joint Pain	2
Nausea	2
Ulcer	2

As mentioned in table-VI, the dietitians perceived diarrhoea and skin allergy to be the main symptom assciated with the gluten-sensitive disease. Other symptoms including joint pain, ulcer, nausea, fatigue, depression, diabetes were also seen as symptom assciated with the gluten-sensitive disease.

Thematic analysis of the perception of dietitians on test to identify gluten-sensitive disease is represented in table-VII

Table- VII

Thematic representation of perception of dietitians on test to identify gluten-sensitive disease

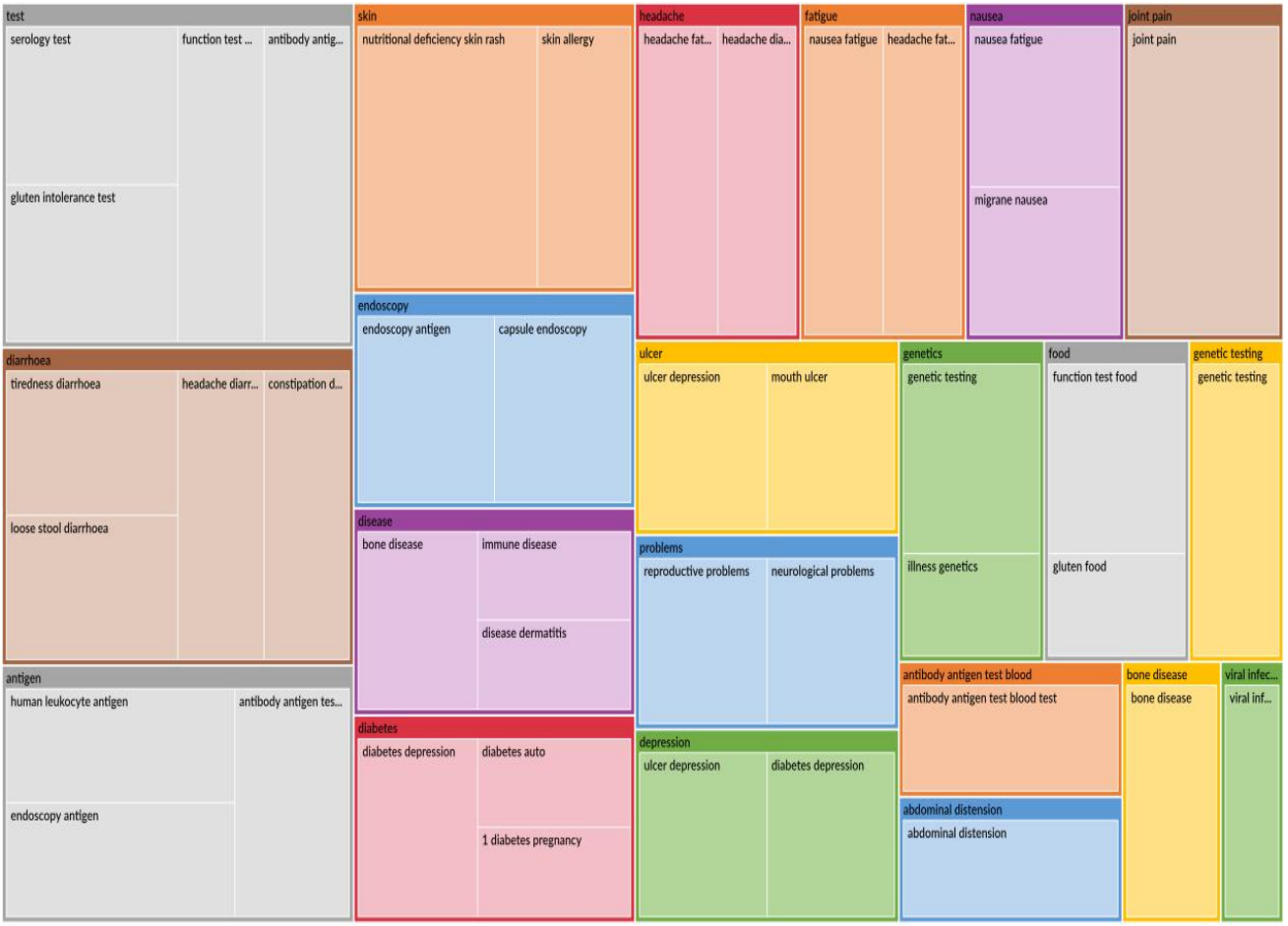
Word	No of times repeated
Antibody test	1
Antigen test	3
Endoscopy	2
Blood Test	1

In table-VII, dietitians mentioned antigen test as the most common test procedure to identify gluten-sensitive disease followed by endoscopy. Antibody test and Blood test were also recommended as test procedure.

Pictorial representation of perception of dietitians on symptoms and test to identify gluten-sensitive disease is represented in figure-9

Pictorial representation of perception of dietitians on symptoms and test to identify gluten-sensitive disease

Figure-9



The picture (figure-9) portrays the responses given by the dietitians in terms of the chart area. This indicates that the test procedure including the serology test, and gluten intolerance test was given more chart area followed by symptoms like diarrhoea, loose stools, headache and constipation, while the least chart area was occupied by an infection like viral infection.overall the figure indicates Diarrhoea as the most common symptom and antibody test as the common common test procedure to identify the gluten-sensitive disease

Thematic analysis of the perception of doctors on symptoms to identify gluten-sensitive disease is represented in the below table-VIII

Table-VIII

Thematic representation of the perception of doctors on symptoms to identify gluten-sensitive disease

Word	No of times repeated
Abdominal Pain	1
Constipation	1
Diarrhoea	2
Skin allergy	5
Fatigue	2
Foggy Brain	2
Skin Rash	2

Based on table-VIII , it is evident that the doctors mentioned skin allergy as most common symptom of gluten-sensitive disease followed by diarrhoea, fatigue, foggy brain , skin rash ,abdominal pain and constipation.

Thematic analysis of the perception of doctors on test to identify gluten-sensitive disease is represented in the below table-IX

Table-IX

Thematic representation of the perception of doctors on test to identify gluten-sensitive disease

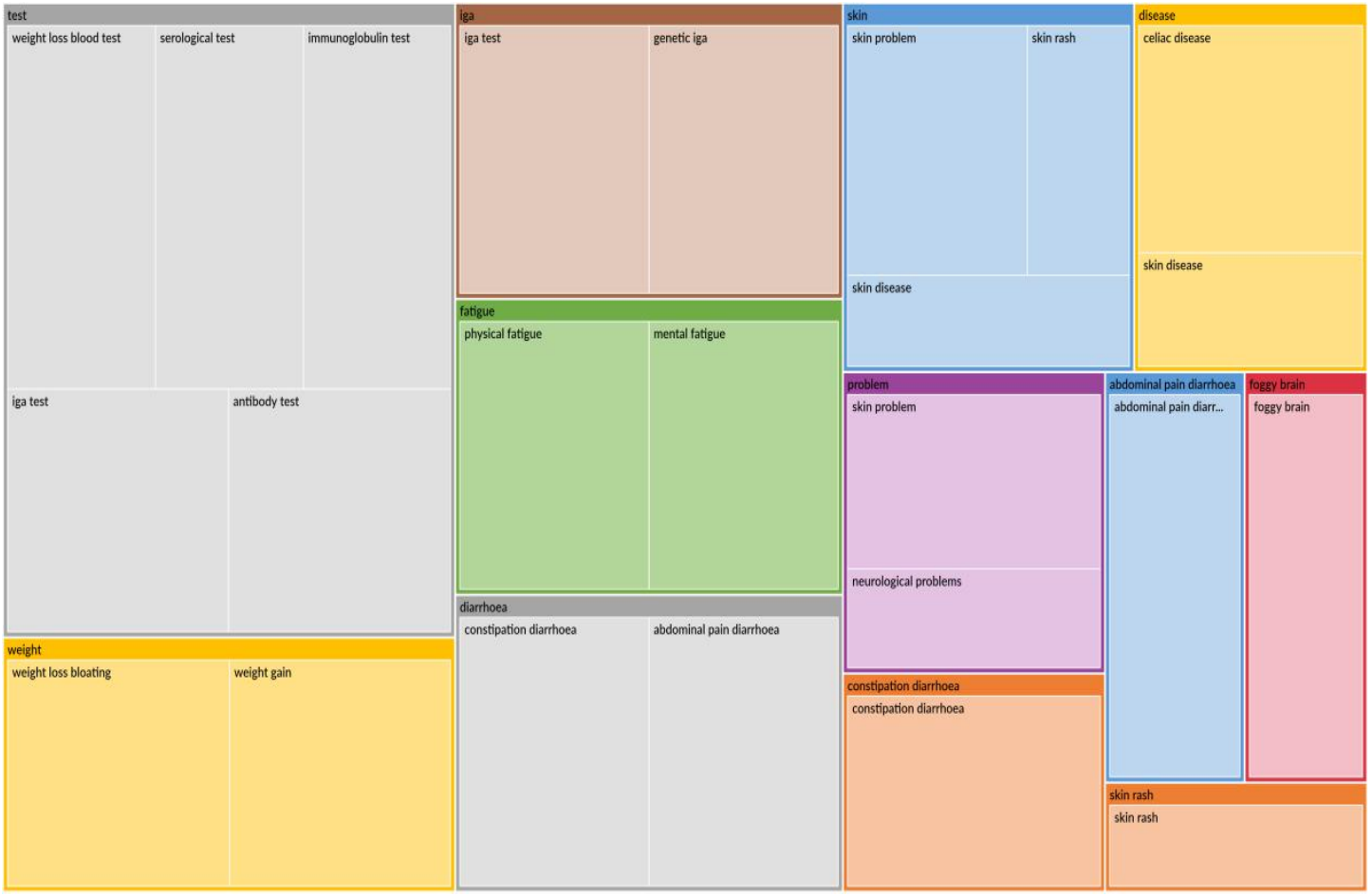
Word	No of times repeated
Blood Test	6
IgA test	2

Table-IX , indicates that the doctors perceived Blood test as the most common test to identify the gluten-sensitive disease followed by IgA test .

Pictorial representation of perception of doctors on symptoms and test to identify gluten-sensitive disease is presented in below figure

Pictorial representation of perception of doctors on symptoms and test to identify gluten-sensitive disease

Figure-10



The picture (figure-10) portrays the responses given by the doctors in terms of the chart area. This indicates that the higher chart area was occupied by test procedures including the IgA test, antibody test, immunoglobulin test, and serological test and the least chart area was occupied by a skin rash. However, both the doctors and dietitians have given preference to the test procedure.

Foods to be recommended for developing gluten-free products:

Perception of dietitians on foods to be recommended for gluten-sensitive disease in the form of the most frequent word is depicted in table-X

Table-X

Perception of dietitians on foods to recommend for gluten-sensitive disease

Word	Length	Count	Weighted Percentage (%)
Fruits	6	26	8.33
Vegetables	10	25	8.01
Egg	3	13	4.17
Dairy products	5	11	3.53
Fish	4	9	2.88
Millet	6	9	2.88
Poultry	7	8	2.56
Rice	4	8	2.56
Legumes	7	7	2.24
Meat	4	7	2.24
Nuts	4	5	1.60
Seeds	5	4	1.28
Soy	3	4	1.28
Cereals	7	3	0.96
Barley	6	2	0.64
Beans	5	2	0.64
Corn	4	2	0.64
Flax seeds	4	2	0.64
Pulses	6	2	0.64

From the table-X it is clear that the dietitians perceived fruits to be the most common food for consumption (8.33%) followed by vegetables (8.01%) and eggs (4.17%).Others foods including dairy products (3.53%) , millet (2.88%) ,rice (2.56%) were also mentioned by most dietitians

Perception of doctors on foods to be recommended for gluten-sensitive disease in the form of the most frequent word is depicted in table-XI.

Table-XI

Perception of doctors on foods to recommend for gluten-sensitive disease

Word	Length	Count	Weighted Percentage (%)
Fruits	6	25	14.71
Millets	7	20	11.76
Vegetables	10	20	11.76
Rice	4	17	10.00
Pulses	6	5	2.94
Dairy products	5	4	2.35
Quinoa	6	4	2.35
Bajra	5	3	1.76
Eggs	4	3	1.76
Millet flours	6	3	1.76
Ragi	4	3	1.76
Jowar	5	2	1.18
Beans	5	1	0.59
Corn	4	1	0.59
Legumes	7	1	0.59
Meat	4	1	0.59
Poultry	7	1	0.59
Rajma	5	1	0.59
Oats	4	1	0.32
Peanut	6	1	0.32
Peas	4	1	0.32

From the table-XI, it is evident that the doctors perceived fruits as the most common food with a weighted percentage of 14.71% and millets and vegetables with a weighted percentage of 11.76% respectively followed by rice (10%)

Factors to be considered for developing gluten-free cookies

Perception of dietitians on factors to be considered for the development of gluten-free cookies in the form of the most frequent word is depicted in a table-.XII

Table-XII

Perception of dietitians on factors to consider for developing gluten-free cookie

Word	Length	Count	Weighted Percentage (%)
Unprocessed	11	2	1.18
Sugar-free	6	1	0.59
Easily digestible	6	1	0.59
Nature foods	6	1	0.59
Omegarich food	5	1	0.59
Low-fat food	5	1	0.59
High Fibre	5	1	0.59

From the table-XII, it is evident that dietitians perceived unprocessed food as the most common factor to develop gluten-free cookies with a weightage percentage of 1.18% followed by sugar-free, low fat, high fibre, omega-rich, easily digestible, natural foods with a weightage percentage (0.59%) respectively.

Perception of doctors on factors to be considered for the development of gluten-free cookies in the form of the most frequent word is depicted in table-.XIII

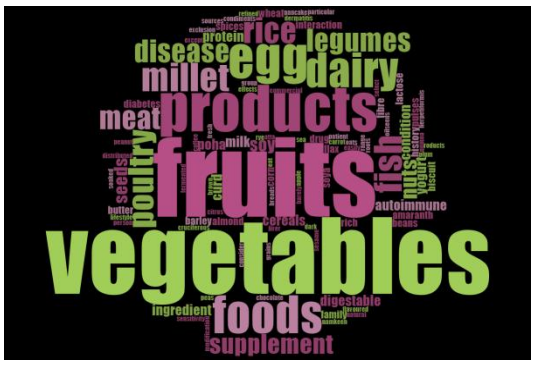
Table-XIII

Perception of doctors on factors to consider for developing gluten-free cookie

Word	Length	Count	Weighted Percentage (%)
Protein-rich food	7	3	0.96
Easily Digestive	10	3	0.96
High Fibre	5	2	0.64
Non refined	7	1	0.32
Fresh foods	5	1	0.32
Fermented foods	9	1	0.32

From table-XIII, it is clear that the doctors perceived protein-rich food and easily digestible food as the most common factor to develop gluten-free cookies with a weightage percentage (of 0.96%) followed by high-fibre food (0.64%). Both dietitians and doctors recommend developing easily digestible, high-fibre foods

Content analysis for the perception of dietitians and doctors on foods and factors to consider for developing gluten-free cookies



Word cloud for the perception of dietitians on foods and factors to consider for developing gluten-free cookies
Figure-11

Word cloud for the perception of doctors on foods and factors to consider for developing gluten-free cookies
Figure-12

The word cloud in figure-11 to analyse perception of dietitians on foods and factors to be considered for developing a gluten-free cookies depicts the word “fruits” was highlighted by most of the dietitians as the foods for developing gluten-free cookies followed by vegetables, eggs, millet, meat and dairy product . “digestible food” was the most highlighted factor to consider for developing gluten-free cookies followed by protein rich food , fibre foods.

The word cloud in figure- 12 depicts the word “fruits” as the most highlighted word by most of the doctors for the foods to be considered for developing gluten-free cookies followed by words like millet ,vegetables,rice and pulses. Doctors perceived “digestible ingredient ” for the factors to be considered for developing a gluten-free cookies followed by protein rich ,fibre rich ingredients.With the help of figure-11 and 12 , thematic analysis was carried out which helped in forming theme .

Table-XIV

Thematic representation of perception of dietitians on foods to consider for developing gluten-free cookies

Words	No of times repeated
Dairy products	2
Fruits	5
Vegetables	1
Millets	2

From the above table-XIV, it is clear that dietitians consider “fruits” for developing a gluten-free cookies followed by millets , dairy products and vegetables.

Table-XV

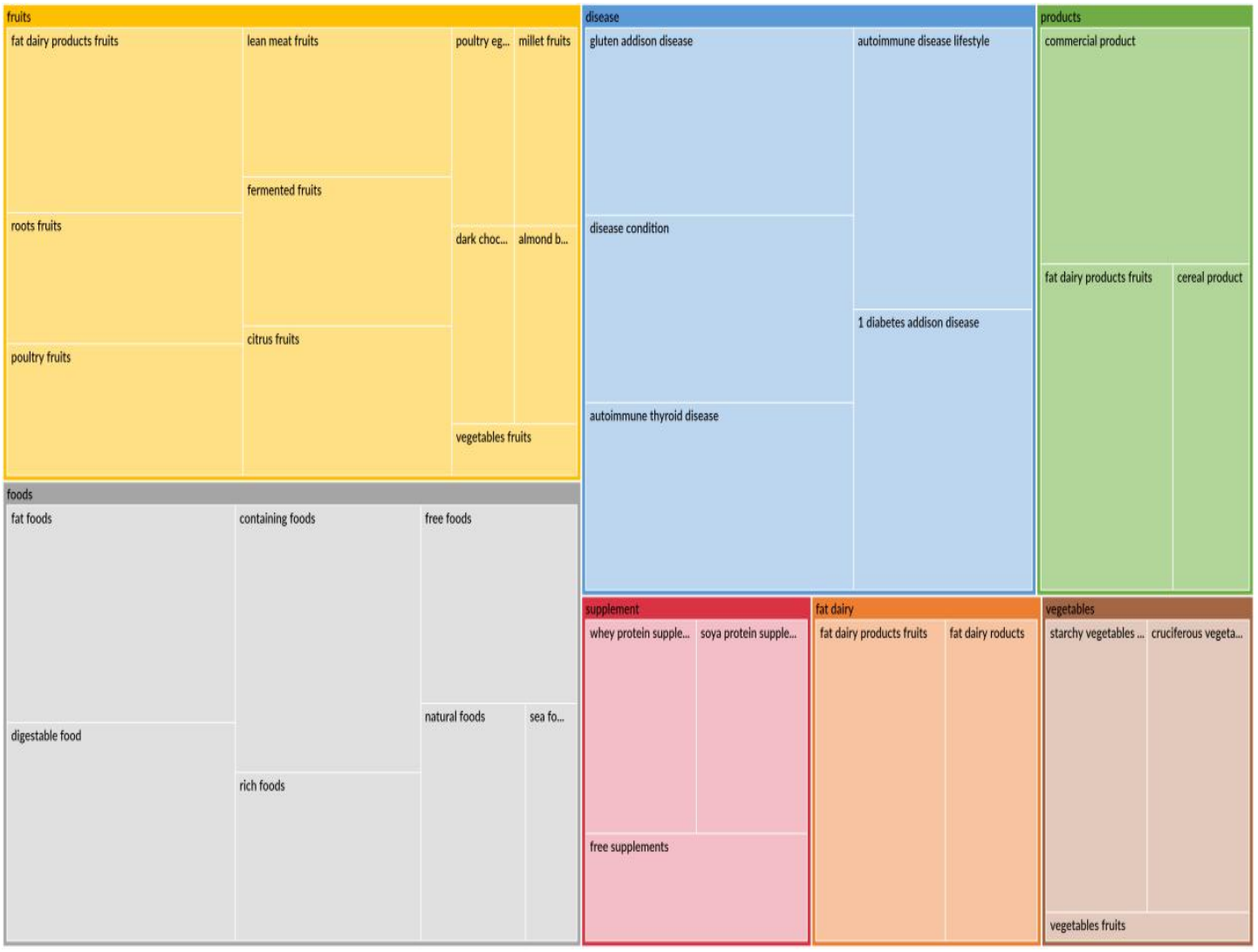
Thematic representation of perception of dietitians on factors to consider for developing gluten-free cookies

Words	No of times repeated
Digestible	4
Protein food	2
Fibre food	2

Based on table -XV it is evident that the dietitians perceived “digestible foods” as the main factors to be considered for the development of gluten-free cookies followed by protein and fibre-rich food .

Figure-13

Pictorial representation of the perception of dietitians on food and factors to be considered for the development of gluten-free cookies



The picture (figure-13) portrays the responses given by the dietitians in terms of the chart area. This indicates that the higher chart area was occupied by fruits that represents that they perceive fruits to be the most preferred food to develop a gluten-free cookie .the figure also indicates that the dietitians prefer to consider factors like easily digestible, fat-free ,protein rich food and fibre rich to develop a gluten-free cookies

Thematic analysis of the perception of doctors on foods and factors to consider for developing gluten-free cookies is represented below in Table-XVI

Table-XVI

Thematic representation of perception of doctors on foods to be considered for developing gluten-free cookies

Words	No of times repeated
Pulses	1
Millets	3
Vegetables	3
Fruits	5
Rice	2

As mentioned in table-XVI, it is clear that the doctors perceived "fruits" as most preferred ingredient to use for developing gluten-free cookie followed by ingredients like millet s and vegetables .They also recommend pulses and rice as they are gluten-free.

Table-XVII

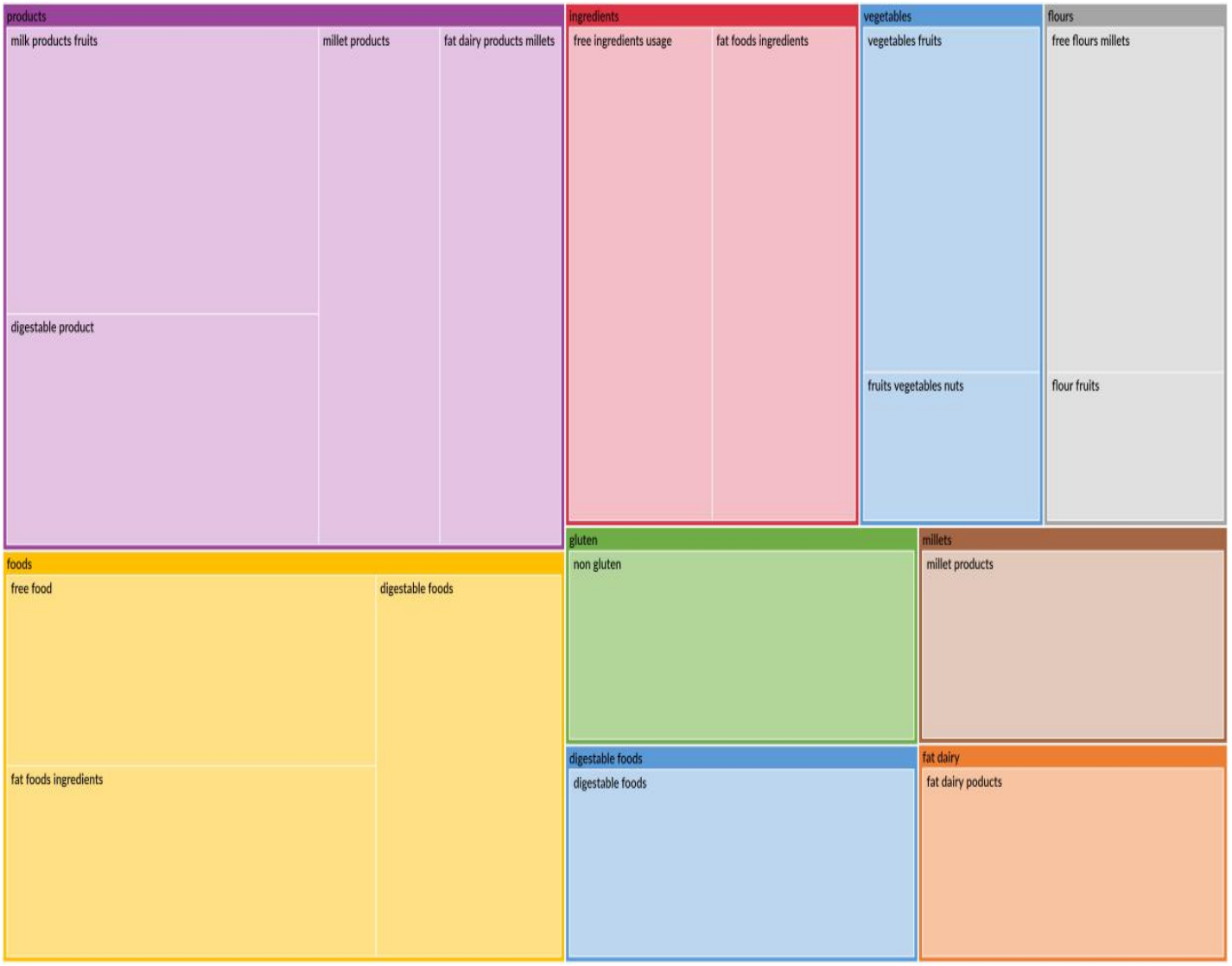
Thematic representation of perception of doctors on factors to be considered for developing gluten-free cookies

Words	No of times repeated
Digestible foods	1
Fat-free	1
Natural Foods	3
Gluten-free Food	5

Based on the table-XVII, it is evident that the dietitian's perceived factors such as gluten-free foods followed by natural food, digestible food and fat-free ingredients to develop a gluten-free cookie.

Pictorial representation of the perception of doctors on food and factors to consider for the development of gluten-free cookies

Figure-14



The picture (figure-14) portrays the responses given by the doctors for perception of foods and factos to be considered for developing a gluten-free cookie in terms of the chart area. This indicates that the higher chart area was occupied by food products including millet products, and milk products followed by food ingredients like easily digestible ingredients, and fat-free ingredients. The last chart area was occupied by dairy fats. Overall both dietitians and doctors perceived fruits, vegetables and millet products as gluten-free ingredients.

PHASE -II

Formulation of gluten-free cookies

Product formulation

Owing to the results and findings of the Phase-I study, Phase II was carried out to formulate a gluten-free cookie. Ingredients that are suggested by both dietitians and doctors during the perception analysis in Phase I were considered for identifying the ingredients for the development of a cookie. Based on the nutraceutical properties and gluten content, waterchestnut flour and Quinoa flour were selected as the main ingredients for the development of gluten-free cookies. Along with this functional ingredients such as sugar, baking soda, butter, soy lecithin powder and xanthan gum powder were used for the development of gluten-free cookies. Thus a total of seven variations of the gluten-free cookie were formulated. They were then evaluated for their organoleptic properties, texture and shelf-life

Mean Acceptability Score of prepared gluten-free cookies

The formulated seven variations of gluten-free cookies were evaluated along with standard unibic cookies as a reference sample for their acceptability by using a nine-point hedonic rating scale. Based on the score obtained, the formulated cookie's were rated as highly acceptable (35-45), acceptable (25-35) and not acceptable (less than 25).The mean acceptability score of the formulated seven variations of gluten-free cookies after the sensory evaluation by 20 semi-trained panel members is tabulated and discussed below.

Table-XVIII

Mean Acceptability Score of formulated gluten-free cookies

Standard / Variation	Appearance	Taste	Texture	Colour	Flavour	Total
VARIATION I (W1) (W:Q-100:0)	8.4±0.88	8.45±0.75	8.35±0.67	8.4±0.66	8.45±0.82	42±2.72
	8.4±0.50	7.85±0.74	7.5±0.82	8.3±0.65	7.6±0.68	39±1.81
VARIATION-II (W2) (W:Q-80:20)	8.45±0.60	8.8±0.52	8.6±.59	8.35±0.67	8.6±.59	42±2.34
	8.65±0.57	8.1±0.78	7.7±0.86	8.4±0.82	7.85±0.87	40±2.88
VARIATION III (W3) (W:Q-60:40)	8.6±0.59	8.65±0.67	8.65±0.48	8.35±0.67	8.7±0.57	42±2.11
	8.25±0.55	8.15±0.58	7.45±0.75	8.4±0.68	8.1±0.71	40±2.20
VARIATION IV (WQ) (W: Q- 50:50)	8.6±0.59	8.65±0.58	8.5±0.68	8.45±0.68	8.55±0.68	42±2.42
	8.65±0.48	8.6±0.59	8.6±0.59	8.55±0.51	8.35±0.58	42±1.75
VARIATION V (Q1)(W:Q-40:60)	8.45±0.68	8.55±0.51	8.6±0.50	8.45±0.68	8.7±0.47	42±2.43
	8.35±0.74	7.15±1.13	7±1.37	7.9±1.11	7.4±1.27	37±5.18
VARIATION VI (Q2) (W:Q-20:80)	8.5±0.68	8.4±0.50	8.6±0.50	8.6±0.50	8.6±0.50	42±2.10
	8.25±0.78	7.55±0.94	7.1±0.78	7.8±0.52	7.55±1.09	38±3.04
VARIATION VII (Q3) (W:Q-0:100)	8.45±0.60	8.6±0.50	8.6±0.50	8.1±0.71	8.55±0.51	42±2.25
	8.45±0.68	7.35±0.81	7.3±0.92	8.2±0.83	7.5±1.0	38±3.12

Standard unibic cookie Variation

In general, all the formulated variation of gluten-free cookies was rated as highly acceptable with a mean score ranging between 37 ± 5.18 and 42±2.72. Out of seven variations, variation IV prepared using 50% waterchestnut flour and 50 % quinoa flour obtained the maximum score of 42±1.75 followed by variation III (40±2.20) made using 60% waterchestnut flour and 40 % quinoa flour and variation II (40±2.88) prepared using 80% waterchestnut flour and 20 % quinoa flour. Shafi, M., et al.,2016 claims that the tendency of waterchestnut flour to absorb water was said to be favourable for baking since hydration enhances the handling of the dough.Thus it can be inferred that gluten-free cookie prepared using waterchestnut flour were more acceptable compared to quinoa flour .The above observation can be attributed by the water absorption tendency of waterchestnut flour resulting in soft dough and cookies Variation V prepared using 40% waterchestnut flour and 60 % quinoa flour scored the least with a mean score of 37±5.18 followed by variation VII (38±3.12) made using 20% waterchestnut flour and 80 % quinoa flour.

Preferential ranking of formulated gluten-free cookies

To ascertain the most preferred cookies of best quality a preference ranking test was carried out.The semi-trained panel members were asked to rank the formulated cookies based on their order of preference. The results were represented in the table below.

Table-XIX

The rank order of developed gluten-free cookie

VARIATION	RANK
VARIATION I (W1) (W: Q-100:00)	4
VARIATION-II (W2) (W: Q-80:20)	3
VARIATION III (W3) (W: Q-60:40)	2
VARIATION IV (WQ) (W: Q- 50:50)	1
VARIATION V (Q1) (W: Q-40:60)	6
VARIATION VI (Q2) (W: Q-20:80)	5
VARIATION VII (Q3) (W: Q-00:100)	7

Variation-IV (WQ) prepared using a combination of 50% waterchestnut flour and 50 % quinoa flour was ranked as the most preferred cookies by the panel members followed by variation-III prepared using 60% waterchestnut flour and 40% of quinoa flour.The gluten-free cookie (VII) prepared using 100% quinoa flour obtained the least rank.Thus it can inferred that gluten-free cookies with equal

proportion of waterchestnut flour and quinoa flour were well acceptable and can have good marketing potential.

Colour analysis of developed gluten-free cookie

Colour values of standard unibic cookies and formulated gluten-free cookie was analysed and the observation are tabulated in table- XX and discussed below

Table-XX

Colour analysis value of developed gluten-free cookies

VARIATION	L*	a *	b *	ΔE*ab
STANDARD	24.55	6.31	9.69	27.13
VARIATION I (W1) (W: Q-100:00)	23.81	6.78	8.85	26.19
VARIATION-II (W2) (W: Q-80:20)	21.36	6.34	9.00	24.03
VARIATION III (W3) (W: Q-60:40)	20.06	7.17	9.52	23.33
VARIATION IV (WQ) (W: Q- 50:50)	23.23	7.49	9.63	26.23
VARIATION V (Q1) (W: Q-40:60)	21.41	6.33	8.35	23.83
VARIATION VI (Q2) (W: Q-20:80)	22.36	6.17	8.08	24.56
VARIATION VII (Q3) (W: Q-00:100)	24.31	5.06	8.10	26.11

According to table-XX, the highest colour score was obtained by the standard product (27.13). Of all variations of developed gluten-free cookies, variation-IV (WQ) prepared using a combination of 50% waterchestnut flour and 50 % quinoa flour was similar in colour to that of standard cookie with a value of 26.23 delta . The least colour score was obtained by variation VII made using 100 % quinoa flour. The colour of the cookie was influenced by the proportion of flour used, along with functional ingredients like sugars, and emulsifiers. According to Cheng and Bhat, 2016, The Maillard reaction between protein and lowering sugars during baking is the main factor in promoting cookie colour. The colour of cookies is influenced by starch caramelization and dextrinization, both of which are enhanced

by heat. Thus it can inferred that the starch content of waterchestnut flour resulted in even dextrinization of cookies.

Texture analysis of prepared gluten-free cookies:

Textural properties of the standard unibic cookies and the formulated gluten-free cookies were analysed and results is represented in table-XXI and figure- 9 followed by discussion

Table-XXI

Texture Analysis of formulated gluten-free cookies

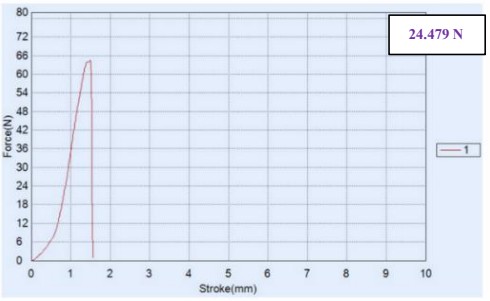
VARIATION	DAY 1	DAY 21
STANDARD-R	76.3853	24.4792
VARIATION I -W1 (W: Q-100:00)	16.8823	23.7627
VARIATION II -W2 (W: Q-80:20)	18.2606	28.8181
VARIATION III-W3 (W: Q-60:40)	49.6558	47.6611
VARIATION IV-WQ (W: Q-50:50)	61.1804	63.0703
VARIATION V-Q1 (W: Q-40:60)	45.8487	47.0207
VARIATION VI-Q2 (W: Q-20:80)	82.5966	19.0143
VARIATION VII-Q3 (W: Q-00:100)	28.5126	20.9497

Figure-15

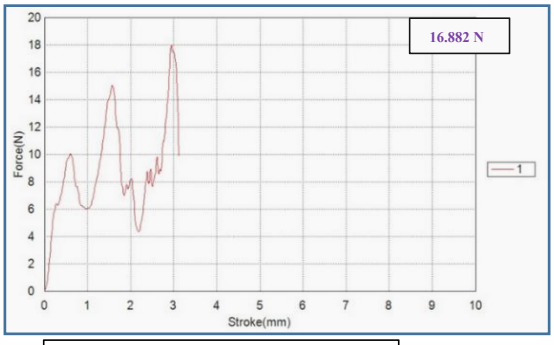
Graphical representation of the textural value of developed gluten-free cookies

STANDARD-R, DAY-1

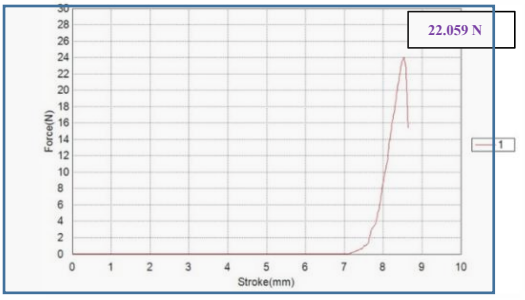
STANDARD-R, DAY-21



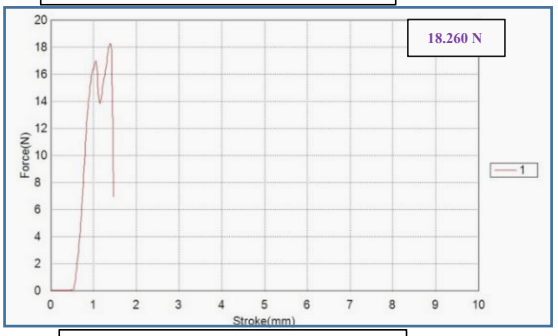
VARIATION I W1 (W: Q -100:0)
DAY-1



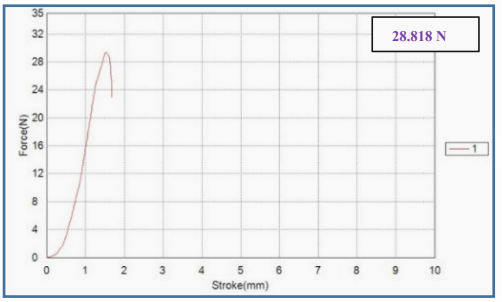
VARIATION I W1 (W: Q -100:00)
DAY-21



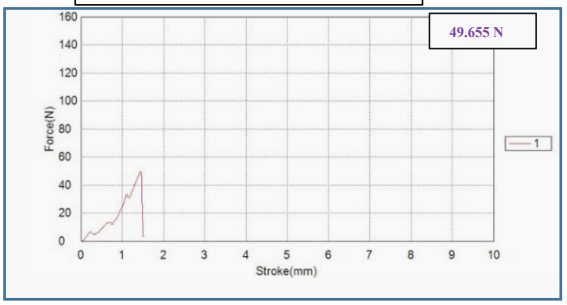
VARIATION II W2 (W: Q -80:20)
DAY-1



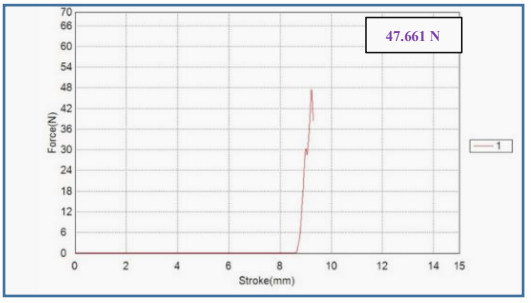
VARIATION II W2 (W: Q -80:20)
DAY-21

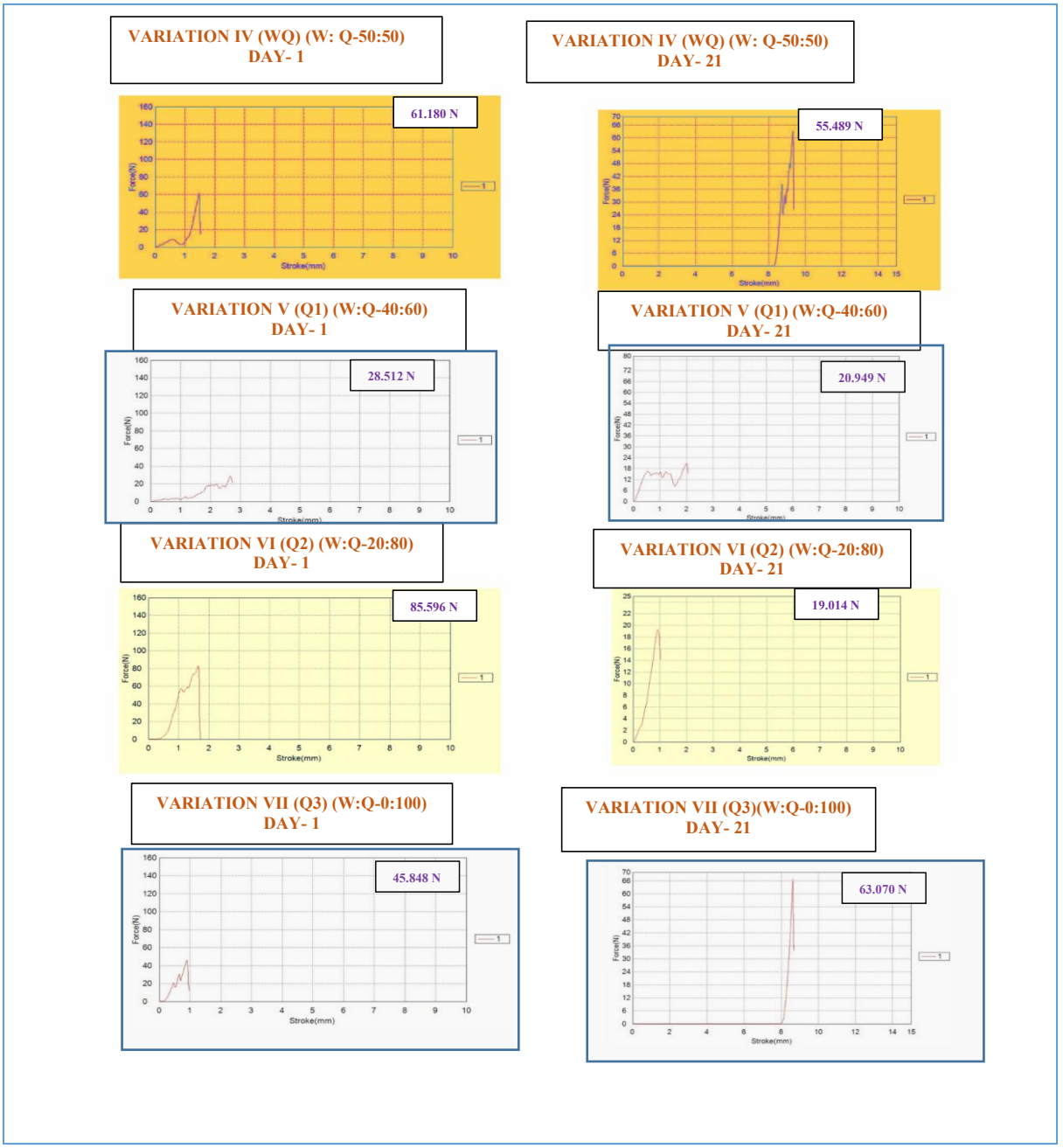


VARIATION III W3 (W: Q -60:40)
DAY-1



VARIATION III W3 (W: Q -60:40)
DAY-21





The above table-XXI and figure-15 illustrate the texture of standard unibic cookies and formulated gluten-free cookies . It was witnessed that the force required to break the standard unibic cookie on the 1st day was 76.3853N which got reduced to 24.4792 N the 21st day indicating that the product sample absorbed atmospheric moisture at a higher rate. However ,the gluten free cookies prepared using 50% of waterchestnut flour and 50% of quinoa flour retained its texture from day one to day 21 with the break force of 63.070 N. The results obtained indicated that variation having equal proportions of both waterchestnut flour and quinoa flour is said to hold its textural characteristics more than other variations. Thus the gluten-free cookies (Variation-IV) interacted less with atmosphere

moisture and retained its texture .Gluten-free cookie (Variation-VI) showed poor textural quality on day 21 with value of 19.0143 N.

Analysis of shelf-life of prepared gluten-free cookie

Analysis of the shelf life of the formulated gluten-free cookies was done by storing the product in an airtight container for 21 days. The obtained result is tabulated in table-XXII and depicted in figure-10

Table-XXII

Mean acceptability score of shelf-life analysis of prepared gluten-free cookies

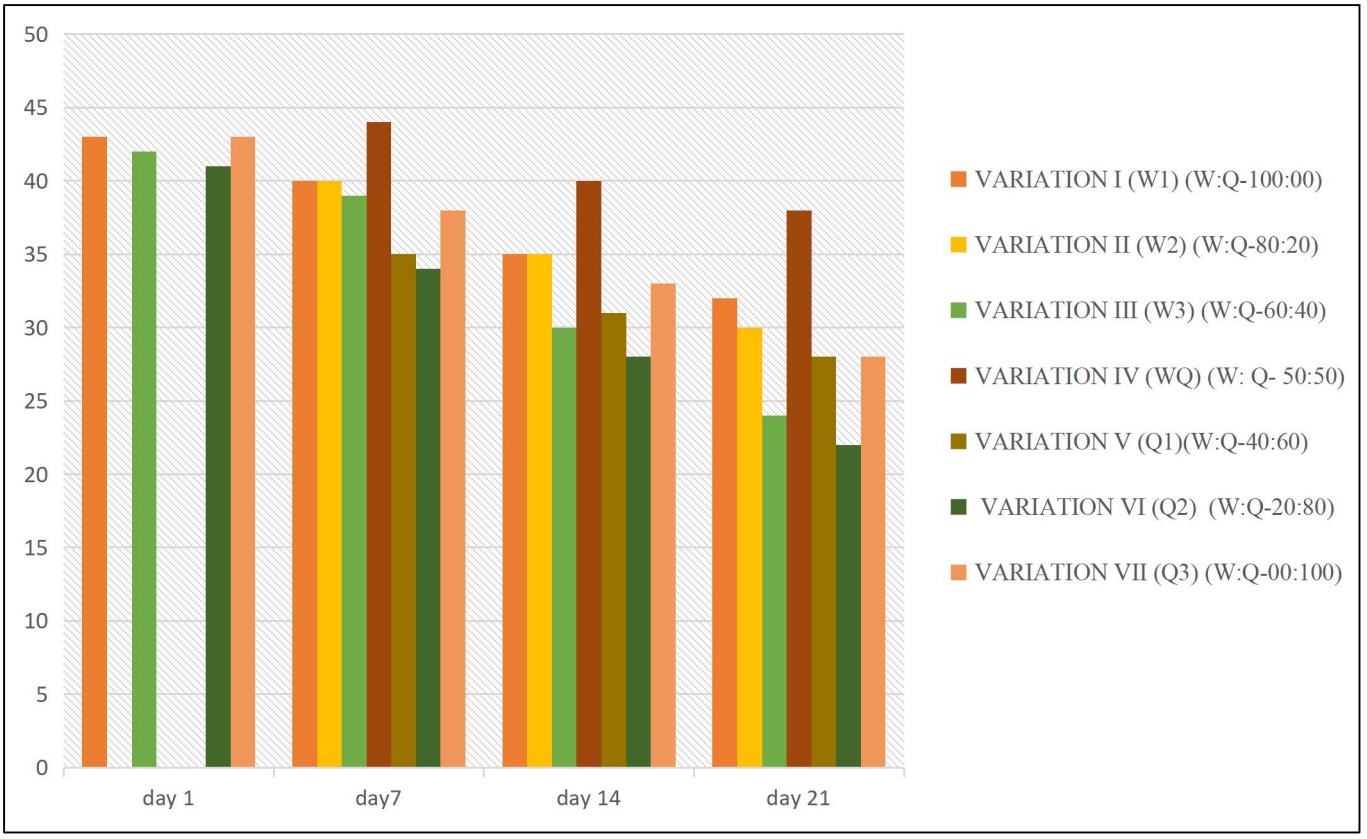
VARIATION	DAY 1	DAY 7	DAY 14	DAY 21
VARIATION I (W1) (W:Q-100:00)	43±0.54	40±0.87	35±0.70	32±1.05
VARIATION II (W2) (W:Q-80:20)	44±0.44	40±0.51	35±0.86	30±0.80
VARIATION III (W3) (W:Q-60:40)	42±0.54	39±0.75	30±1.24	24±0.95
VARIATION IV (WQ) (W: Q- 50:50)	45	44±0.16	40±0.70	38±0.58
VARIATION V (Q1)(W:Q-40:60)	42±0.89	35±0.92	31±1.30	28±1.11
VARIATION VI (Q2) (W:Q-20:80)	41±1.09	34±1.25	28±1.09	22±0.86
VARIATION VII (Q3) (W:Q-00:100)	43±0.54	38±0.36	33±0.54	28±1.07

The mean score obtained from the shelf-life analysis indicated that the average score ranged between 22±0.95 to 38±0.58. Variation IV (WQ) made using 50 % waterchestnut flour and 50 % quinoa flour since the product was holding its sensory attributes. The least shelflife score was obtained by variation-VI (Q2) prepared using 20% waterchestnut flour and 80% quinoa flour. Thus the gluten-free cookie (Variation-IV) was having a high shelf life period compared to the other variations and can have a good marketing potential.

A graphical representation of the shelf-life analysis of developed gluten-free cookies is illustrated in figure-16

Graphical representation of mean score for shelf-life analysis of developed gluten-free cookies

Figure-16



Nutrient analysis of prepared gluten-free cookie

Along with the standard unibic cookies, the nutrient analysis of selected variation of gluten-free cookies that had the maximum mean acceptability score (42±1.75)namely Variation-IV (WQ) made using 50 % waterchestnut flour and 50 % quinoa flour was selected for nutrient analysis .

Nutrient analysis per package of standard unibic cookies and selected gluten-free cookies are given in the following tables.

Table-XXIII

Nutrient analysis of gluten-free cookies

NUTRIENT (100 g)	STANDARD		VARIATION-IV(WQ) (W:Q- 50:50)	
	100 g	**20 g**	**100 g**	**20 g**
Moisture (g/100g)	6.23	1.25	5.23	1.04
Total ash (g/100g)	4.92	1	5.02	1
Fat (g/100g)	19.2	4	16.1	3
Total protein (g/100g)	5.23	1	6.23	1.2
Carbohydrate (g/100g)	64.42	13	67.42	13
Energy (kcal/100 g)	453.2	90	442.1	88
Calcium (mg/100 g)	46.2	9	41.6	8
Iron (mg/100 g)	4.26	0.8	6.23	1.24
Crude fibre (g/100g)	6.22	1.2	7.11	1.4
Total sugar (g/100g)	39.2	8	38.1	7
Gluten (g/100g)	7.23	1.4	3.14	0.6

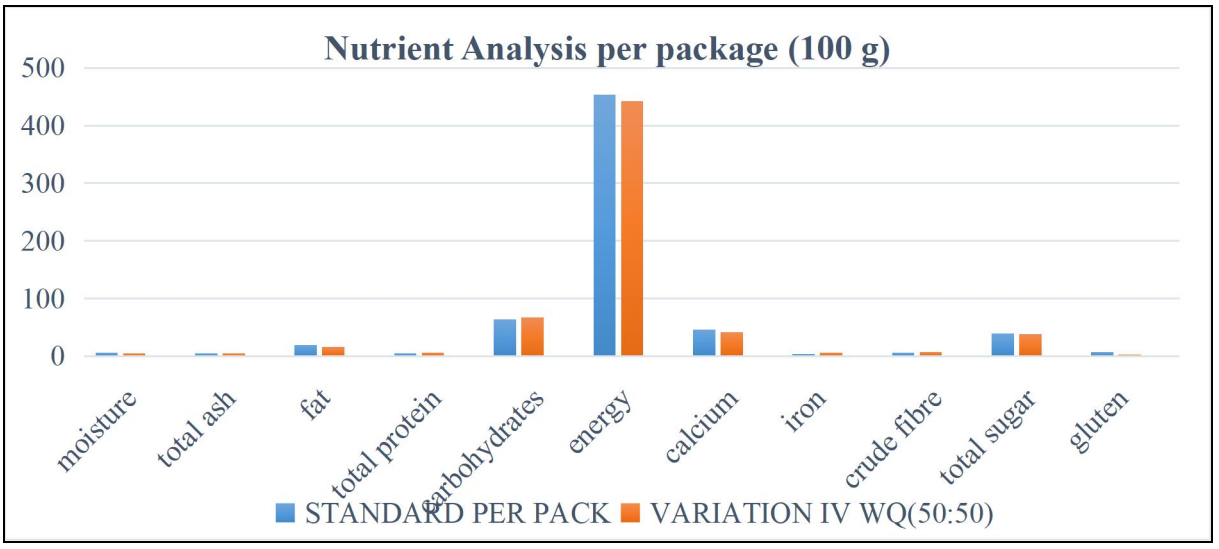
In comparison to the nutrient composition of standard unibic cookies, the selected variation of gluten-free cookie prepared using 50 % waterchestnut flour and 50 % quinoa flour was said to be superior in nutrients like total protein (6.23 g/100 g), iron(6.23 mg/100 g) and fibre (7.11 g/100 g).Since waterchestnut flour is a good source of dietary fibre (4-7%) (Rajkumar, P., & Rajithasri, M.,2022) and quinoa flour is a good source of essential amino acids (protein), dietary fibre and iron (Hussain, M. I. et al., 2021).The formulated gluten-free cookie can be suggested as a healthy alternative

snacks for all age groups .Also the gluten content of the 100 gram of cookies was found to be 3.14 gram that was comparatively lower than the standard product.

Nutrient analysis per package of standard unibic cookies and selected gluten-free cookies is graphically represented in Figure- 17

Graphical representation of nutrient analysis of developed gluten-free cookies(100 g)

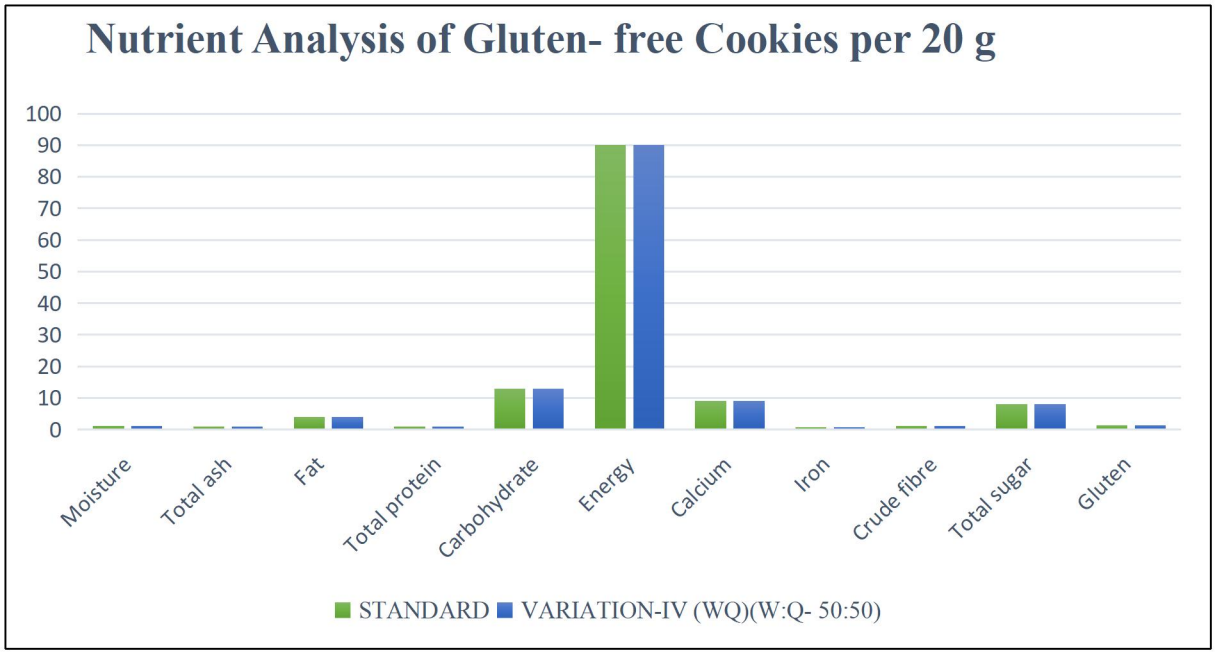
Figure-17



Nutrient analysis per serving of standard unibic cookie and the selected gluten-free cookie is graphically represented in Figure- 18

Graphical representation of nutrient analysis of developed gluten-free cookies(20 g)

Figure-18



Nutraceutical analysis of prepared gluten-free cookie

Nutraceutical analysis of standard unibic cookies and best variation of gluten-free cookies was carried out using standard procedure.The results are tabulated and discussed below

Table-XXIV

Nutraceutical analysis of gluten-free cookies

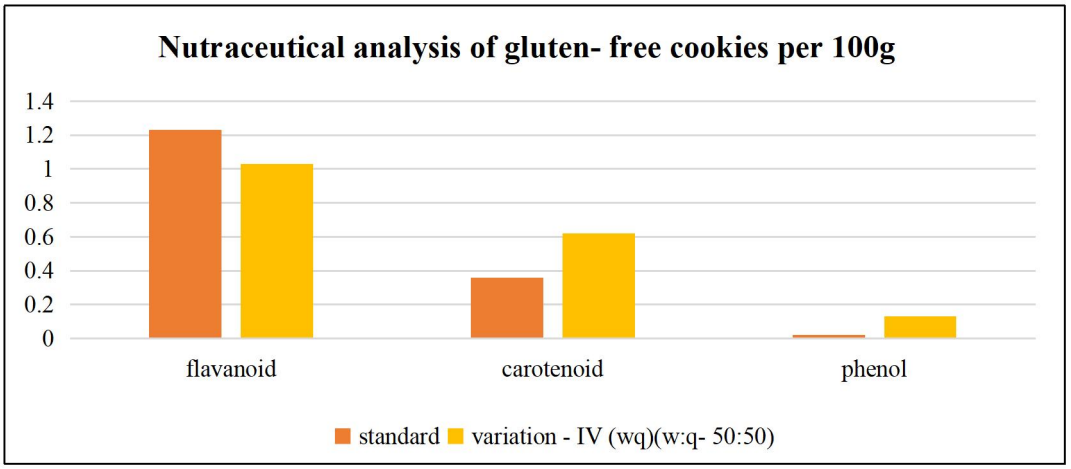
NUTRIENT (100 g)	STANDARD		VARIATION-IV (WQ)(W:Q- 50:50)	
	100 g	20 g	100 g	20 g
Flavonoid (mg /100 g)	1.23	0.2	1.03	0.2
Carotenoid (mg /100 g)	0.36	0.07	0.62	0.12
Phenol (mg /100 g)	0.02	0	0.13	0.02

Table-XXIV represents the nutraceutical attributes of a selected variety of cookies and standard unibic cookies. The result indicated the flavonoid content was superior in the standard product compared to the developed variation (1.03 mg/100 g) whereas the carotenoid (0.62 mg/100 g) and phenol (0.13 mg/100 g) content were superior in the variation-IV cookies.

Nutraceutical analysis per package of standard unibic cookie and the selected gluten-free cookie is graphically represented in figure-19

Graphical representation of nutraceutical analysis of developed gluten-free cookies(100g)

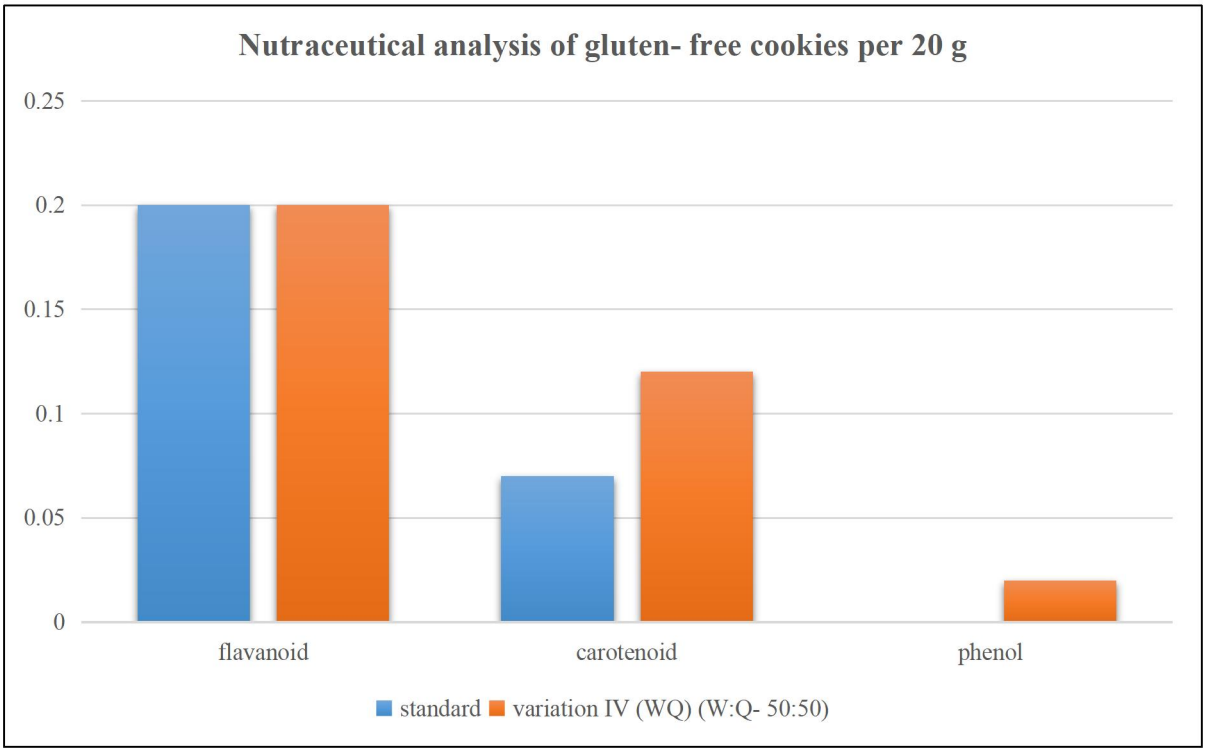
Figure-19



Nutraceutical analysis per serving of standard unibic cookie and the selected gluten-free cookie is graphically represented in figure-20

Graphical representation of nutrient analysis of developed gluten-free cookies(20 g)

Figure-20



COST CALCULATION OF FORMULATED GLTUEN-FREE COOKIE

The cost per pack and serving of the selected variant of the gluten-free cookie was calculated considering the factors like food cost, labour cost and overhead expenses into account. The cost per serve and pack is mentioned below

Table -XXV

Cost estimation of developed gluten-free cookie

QUANTITY (G)	COST (Rs)
Per package (100 g)	50
Per serve (20 g)	10

SUMMARY AND CONCLUSION

V SUMMARY AND CONCLUSION

Bakery product consumption is increasing daily due to its nutritional value and viability for use in feeding programmes and emergency scenarios like earthquakes. Many studies on cereal science and technology offer an understanding of the research activities used to study bakery goods. Cookies are one of the most popular and widely consumed produced foods in India. Due to their appealing qualities including a longer shelf life, a variety of tastes and textures, as well as widespread consumption, cookies are a flexible snack in the food industry and one of the bakery products. Market sellers are concentrating on producing clean-label cookies as a result of growing consumer knowledge of the health implications of certain substances that are being added to them. Currently, cookies are used as everyday foods, upscale presents, snacks, baby foods, dietary products, dog and cat feeds, and embellished things with the addition of chocolate, cream, almonds, and other flavours. As a result, there is a significant opportunity to enhance the nutritional value to satisfy the enormous customer demand for cookies made with healthier components as well as to make them more palatable and nutritious. Additionally, as a result of rising customer demand, the number of coffee shops around the world has expanded which resulted in increased cookie consumption The market for cookies was estimated to be worth US$ 35.95 program billion in 2021, and from the year 2021 to 2029, total revenue is anticipated to increase by 5.5%, or roughly US$ 55.17 billion. One of the world's top producers of cookies is India. In India, the growth rate of the cookie market is expected to reach USD 5,151.2 million in 2020 at a CAGR of 12.4% and by 2027, the market is anticipated to grow to USD 11,792.3 million. The availability of diverse types of cookies, rising disposable incomes, and growing packaged and convenience food consumption are some of the reasons that are driving the Indian biscuits industry.

Gluten is a protein compound that is present in cereals like wheat (gliadins and glutenins), rye (secalins and secalinins), oat (avenins and avenalins) and barley(hordeins and hordenins) .prolamins and glutelins are the ethanol-soluble proteins that makeup gluten. These proteins give the dough the flexibility needed for leavening and shaping because they are high in glutamine and proline residues but are resistant to digestion by human intestinal proteases. However, ingesting some wheat substances such as gliadins, glutenins, fermentable oligosaccharides, disaccharides, monosaccharides and polyols may cause various problems. These are known as Gluten Related Disorders. These include celiac disease wheat allergy and non-celiac gluten sensitivity.

The study entitled “DEVELOPMENT OF VALUE-ADDED GLUTEN-FREE COOKIE FOR GLUTEN-SENSITIVE DISEASE” was framed with the objective of, identifying the perception on gluten sensitive disease by Dietitians and Doctors and Formulating gluten-free cookies.

The current investigation was carried out in two phases -Perception of gluten-sensitive disease among dietitians and doctors in Coimbatore was carried out as the first phase. Three multispeciality hospitals with well-established dietary departments were selected for the conduct of the study based on convenience sampling. Based on purposive sampling Dietitians (17) and Doctors (13), a total of 30 members were selected for the study. An open-ended questionnaire comprising 9 questions in total was asked out fill by the subjects which were later analyzed using NVivo software

In the second phase of the study, the Formulation of gluten-free cookies was carried out. Ingredients based on the response collected from dietitians and doctors were used for developing the product. Selected waterchestnut flour and Quinoa flour were then combined in different ratios to develop seven variations of cookies. Functional ingredients including sugar, baking soda, soy lecithin powder and xanthan gum powder were also incorporated into the product. The formulated variations were then compared to the standard unibic cookies taken as a reference sample for the study.

Organoleptic evaluation using 20 semi-trained panel members was conducted. Following the organoleptic evaluation, a preference ranking test was conducted by the investigator, to identify the best variation .

The variations were then studied for their shelf life properties for 21 days by the investigator where the investigator observe and taste the product for its appearance, taste, texture, colour and flavour and score them using a 9-point hedonic rating scale for 21 days and analyse the shelf life characteristics of the product

Textural properties of both the standard unibic cookies and the formulated seven variations of cookies were analysed using a textural analyser on day 1, day 7, day 14 and day 21 from the day of baking. A colour reader is used to compare the colour of the developed cookies to the standard unibic cookie. The variation that scored the maximum in all attributes including -organoleptic evaluation, preference ranking test, colour reader, texture analysis and shelf life study was selected and analysed for its nutrient and nutraceutical properties and packed and labelled.

The salient findings of the study were:

- Dietitians were more knowledgeable in terms of gluten-sensitive disease, gluten-free foods and diets for gluten-sensitive patients when compared to Doctors.
- Dietitian perceived diarrhoea, bloating and abdominal pain as major symptoms of gluten-sensitive disease with a weighted percentage of 5.23%,4.18% and 3.48% respectively.
- Doctors perceived constipation as the most common symptom(7.30%) followed by diarrhoea (6.18%) and skin disease (6.18%).
- Both dietitians and doctors perceived bloating as a symptom of gluten-sensitive disease with a weightage of 4%.
- Dietitians perceived blood tests as the most common test for identifying gluten-sensitive disease at a weightage of 3.48% followed by antigen test (1.74%) and biopsy (1.74%)
- Doctors perceived the IgA test (3.37%) as the frequently preferred test followed by the Immunoglobulin test (0.56%), serological test (0.56%) and antibody test (0.5%)
- Both dietitians and doctors recommend an Antibody test and IgA test for identifying gluten-sensitive disease
- Test procedure including the serology test, and gluten intolerance test was given more chart area by the dietitians followed by symptoms like diarrhoea, loose stools, headache and constipation
- According to the perception of doctors higher chart area was occupied by test procedures including the IgA test, antibody test, immunoglobulin test, and serological test
- Dietitians perceived fruits to be the most common food for consumption (8.33%) followed by vegetables (8.01%) and eggs (4.17%).Others foods including dairy products (3.53%) , millet (2.88%) ,rice (2.56%) were also mentioned
- Doctors perceived fruits as the most common food with a weighted percentage of 14.71% and millets and vegetables with a weighted percentage of 11.76% respectively followed by rice (10%)
- Dietitians perceived unprocessed food as the most common factor to develop gluten-free cookies with a weightage percentage of 1.18% followed by sugar-free, low fat, high fibre, omega-rich, easily digestible, natural foods with a weightage percentage (0.59%)
- Doctors perceived protein-rich food and easily digestible food as the most common factor to develop gluten-free cookies with a weightage percentage (of 0.96%) followed by high-fibre food (0.64%).
- Both dietitians and doctors recommend to develop easily digestible, high-fibre food
- Responses of doctrs indicates that the higher chart area was occupied by food products including millet products, and milk products followed by food ingredients like easily digestible ingredients, and fat-free ingredients

- Both dietitians and doctors perceived fruits, vegetables and millet products as gluten-free ingredients.
- Variation IV (WQ) prepared using 50% waterchestnut flour and 50 % quinoa flour obtained the maximum score of 42±1.75
- Gluten-free cookies with equal proportion of waterchestnut flour and quinoa flour (Variation-IV) was ranked the highest and can have good marketing potential
- Highest colour score was obtained by the standard product (27.13). Of all variations of developed gluten-free cookies, variation-IV (WQ) prepared using a combination of 50% waterchestnut flour and 50 % quinoa flour was similar in colour to that of standard cookie with a value of 26.23 delta
- The gluten-free cookies prepared using 50% of waterchestnut flour and 50% of quinoa flour retained its texture from day one to day 21 with the break force of 63.070 N
- The shelf-life analysis indicated that the average score ranged between 22±0.95 to 38±0.58. Variation IV (WQ) made using 50 % waterchestnut flour and 50 % quinoa flour was selected since the product was holding its sensory attributes with mean score of 38±0.58. The least shelflife score was obtained by variation-VI (Q2) prepared using 20% waterchestnut flour and 80% quinoa flour. Thus the gluten-free cookie (Variation-IV) was having a high shelf life period compared to the other variations and can have a good marketing potential.
- Variation-IV (WQ) of gluten-free cookies made using 50 % waterchestnut flour and 50 % quinoa flour had the maximum mean acceptability score of 42±1.75 was selected for nutrient analysis.
- Selected variation of gluten-free cookie Variation-IV (WQ) prepared using 50 % waterchestnut flour and 50 % quinoa flour was said to be superior in nutrients like total protein (6.23 g/100 g), iron(6.23 mg/100 g) and fibre (7.11 g/100 g)
- The formulated gluten-free cookie can be suggested as a healthy alternative snacks for all age groups.
- The gluten content of the 100 gram of cookies was found to be 3.14 gram that was comparatively lower than the standard product.
- The flavonoid content was superior in the standard product compared to the developed variation (1.03 mg/100 g) wheareas carotenoid (0.62 mg/100 g) and phenol (0.13 mg/100 g) content were superior in the variation-IV (WQ) cookies prepared using 50 % waterchestnut flour and 50 % quinoa flour than the standard cookies.

Conclusion:

Development of Gluten-free cookie is the need of the hour for food processing industry. Replacement of gluten containing ingredient with gluten-free ingredient can serveas an healthy alternate not only for individual with gluten sensitive diseaes but also for idividuals of all age group. apart from the functional benefits such as low calorie ,gluten free iron and fiber rich antioxidant dense, anti-inflammatory and anti-diabetic formulated gluten free cookies can be a prospective product in food processing industry.

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APPENDICES

APPENDIX- I

ETHICAL CLEARANCE FROM INSTITUTIONAL HUMAN ETHICS COMMITTEE

INSTITUTIONAL HUMAN ETHICS COMMITTEE

Avinashilingam

Institute for Home Science and Higher Education for Women
(Deemed to be university under Category 'A' by MHRD, Estd. u/s 3 of UGC Act 1956) Re-accredited with 'A^{++}' Grade by NAAC.
Recognised by UGC Under Section 12 B
Coimbatore- 641043, Tamil Nadu, India

05.01.2023

Chairman
Dr. Sudha Ramalingam
Director – Research and Innovation
Professor- Community Medicine,
PSG Institute of Medical Sciences
& Research, Coimbatore.

Member Secretary
Dr A Thirumani Devi
Professor
Department of Food Science
and Nutrition

Members
Mr. K Arulmoli (Legal Expert)
Dr. Subashini K.Sripathi
Dr. A Saraswathy(Medical Officer)
Ms. D. Kavitha
Dr. A R Sudamani Ramasamy
Dr. G. Victoria Naomi
Dr. Judith Justin
Dr. Anitha Subash
Dr. K Sampath Rani

To
Ms. Aarthi Swetha, M.
Department of Food Service Management and Dietetics
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore- 641043

Dear Aarthi Swetha,

Ref: Your proposal No. IHEC/22-23/FSMD-01 entitled "Development of Value-Added Gluten-free Cookies for Gluten-Sensitive Diseases" submitted for approval of IHEC 21.11.2022

The Institutional Human ethics Committee of our University hereby grants approval to your research proposal No. IHEC/22-23/FSMD-01 entitled "Development of Value-Added Gluten-free Cookies for Gluten-Sensitive Diseases" submitted by you. The Approval number for the same is AUW/IHEC/FSMD- 22-23/XPD-01.

We wish you all the best in your research endeavours.

Regards

5.1.23

Dr. A Thirumani Devi
Member Secretary

Institutional Human Ethics Committee

APPENDIX- II

OPEN ENDED QUESTIONNAIRE

GENERAL INFORMATION

Name: Date:

Age: ☐ 20-30 years ☐ 30-40 years ☐ 40-50 years ☐ 50-60 years

Gender : ☐ Male ☐ Female

Clinic /hospital name:

Profession : ☐ Doctor ☐ Dietitian/Nutritionist

Qualification :

Years of experience : ☐ 1-10 years ☐ 10-20 years ☐ 20 -30 years ☐ > 30 years

Please give us your response to the following questions regarding gluten sensitivity to the best of your knowledge

1. Can you sensitize me on the difference between gluten sensitivity and celiac disease?
2. What are the classical symptoms normally observed in gluten sensitive patient
3. What are the other unusual symptoms that you have observed in patients experienced who are gluten sensitive?
4. What are the factors that commonly triggers gluten sensitivity in a person?
5. What test do you commonly suggest to identify gluten related disease
6. What are the foods that are normally recommended by you to gluten sensitive patients?
7. Do you suggest any branded products for gluten-sensitive patients?
8. What are the factors to be considered to develop a food product for gluten sensitivity?
9. Can you give me the names of the ingredients that are gluten free and rank them according to your preference

APPENDIX III

HEDONIC RATING TEST

TITLE OF STUDY - DEVELOPMENT OF VALUE ADDED GLUTEN FREE COOKIE FOR GLUTEN SENSITIVE DISEASE

Name:_____ Date:_____

Product:_____

Taste these samples and check how much you like or dislike each one . Use the appropriate scale to show your attitude by checking at the point that best describes your feelings about the sample.

	STANDARD	**VARIATION**
Appearance		
Taste		
Texture		
Colour		
Flavour		
Overall Acceptability		

1- Dislike extremely

2- Dislike very much

3- Dislike moderately

4-Dislike slightly

5- Neither like nor dislike

6- Like slightly

7- Like moderately

8- Like very much

9- Like extremely

Appearance	S	I	E
Taste	S	I	E
Texture	S	I	E
Colour	S	I	E
Flavour	S	I	E

Signature_____

Date _____

APPENDIX IV

PREFERENCE RANKING TEST

NAME:_____

DATE:_____

Please taste and rank the samples from most preferred (1) to the least preferred (7)

SAMPLES

VARIATION-I (W1) _____

VARIATION-II (W2) _____

VARIATION-III (W3) _____

VARIATION-IV (WQ) _____

VARIATION-V (Q1) _____

VARIATION-VI (Q2) _____

VARIATION-VII (Q3) _____

Signature:_____

Date:_____

APPENDIX -V

DETERMINATION OF MOISTURE CONTENT

PROCEDURE:

- Dry the empty dish and lid in the oven at 105°C for 3 hours and transfer to desiccator to cool. Weight the empty dish and lid.
- Weigh about 3 g of sample to the dish. Spread the sample to the uniformity.
- Place the dish with sample in the oven. Dry for 3 hours at 105°C.
- After drying, transfer the dish with partially covered lid to the desiccators to cool.
- Reweigh the dish and is dried sample.

CALCULATION:

$$\textbf{Moisture (\%)} = \frac{(W1 - W2)}{W1} \times 100$$

Where,

W1 = Weight (g) of the sample before drying

W2 = Weight (g) of the sample after drying

APPENDIX -VI

DETERMINATION OF TOTAL ASH

PROCEDURE:

- Place the crucible and lid in the furnace at 550°C overnight to ensure that impurities on the surface of crucible are burned.
- Cool the crucible in the desiccator for 30 minutes. Weigh the crucible and lid to 3 decimal places.
- Weigh about 5 g of sample into the crucible. Heat over low flame Bunsen flame with lid half covered.
- Heat at 550°C overnight. During heating, do not cover the lid. Place the lid after completed heating to prevent loss of fluffy ash. Cool down in the desiccator.
- Weight the ash with crucible and lid when the sample turns to grey. If not, return the crucible and lid to the furnace for further asking.

CALCULATION:

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of the sample}} \times 100$$

APPENDIX -VII

DETERMINATION OF TOTAL FAT

METHOD – Soxhletmethod **REAGENT –** Ether

PROCEDURE

- Weigh, accurately 5-10 g (W1) of dry sample into a thimble and keep a cotton plug on top of it
- Place the thimble in a Soxhlet apparatus and add ½ volumes of Ether into a pre-weighed flat- bottom flask (W2) and distilled for 16 hours (Cool the apparatus and filter the solvent into a pre- weighed conical flask (W2)
- Rinse the flask of the apparatus with small quantities of Ether and then added washings to the above flask)
- Remove Ether by evapouration and dried the flask with fat at 80-100 C, cool in a desiccator and weigh (W3)

CALCULATION

$$\text{Fat content (g/ 100\%)} = \frac{(W3-W2) \times 100}{W1} = X$$

Where, W1- Weight of dry matter taken for extraction

W2- Weight of flat bottom flask, W3- Weight of flask with fat

APPENDIX-VIII

DETERMINATION OF TOTAL PROTEIN

METHOD – Lowry's method

REAGENTS

- 2% Sodium carbonate in 0.1 N Sodium hydroxide (Reagent A)
- 0.5% Copper sulphate (CuSO4.5H2O) and 1% Potassium sodium tartarate in 100 ml distilled water (Reagent B)
- Alkaline copper solution: Mix 50 ml of A and 1 ml of B prior to use (Reagent C)
- Folin-Ciocalteau Reagent
- Protein Solution (Stock Standard) - Weigh accurately 50 mg of bovine serum albumin and dissolve in distilled water and make up to 50 ml in a standard flask
- Working Standard- Dilute 10 ml of the stock solution to 50 ml with distilled water in a standard flask. 1 ml of this solution contains 200 µg protein

PROCEDURE

- Extraction of Protein from sample: Extraction is usually carried out with buffers used for the enzyme assay. Weigh 500 mg of the sample and grinded well with a pestle and mortar in 5-10 ml of the buffer. Centrifuged and used the supernatant for protein estimation
- Pipette 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard into a series of test tubes
- Pipette 0.1 ml and 0.2 ml of the sample extract in two other test tubes
- Make up the volume to 1 ml in all the test tubes. A tube with 1 ml of water serves as the blank
- Add 5 ml of reagent C to each tube including the blank. Mix well and allow standing for 10 minutes
- Then add 0.5 ml of reagent D, mix well and incubated at room temperature in the dark for 30 minutes. Blue color is developed
- Take the readings at 660 nm
- Draw a standard graph and calculate the amount of protein in the sample and express the amount of protein in mg/g or 100 g sample.

APPENDIX-IX

DETERMINATION OF TOTAL CARBOHYDRATE

METHOD – Anthrone method

REAGENTS

- 2.5N HCl
- Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice-cold 95% Sulphuric acid. Prepare fresh before use
- Standard Glucose (stock): Dissolve 100 mg in 100 ml distilled water
- Working standard: 10 ml of stock diluted to 100 ml with distilled water. Store refrigerated after adding a few drops of Toluene

PROCEDURE

- Weigh 10-100 mg of the sample in to a boiling tube
- Hydrolyze by keeping it in a boiling water bath for 3 hours with 5 ml of 2.5 N HCl and cool to room temperature
- Neutralize with solid Sodium carbonate until the effervescence ceases
- Make up the volume to 100 ml and centrifuge
- Collect the supernatant and take 0.5 and 1 ml aliquots for analysis
- Prepare the standards by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard, '0' serves as blank
- Make up the volume to 1 ml in all the tubes including the sample tubes by adding distilled water
- Then add 4 ml of Anthrone reagent
- Heat for 8 minutes in a boiling water bath
- Cool rapidly and read the green to dark green color at 630 nm
- Draw a standard graph by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis
- From the graph calculate the amount of carbohydrate present in the sample tube

APPENDIX-X

DETERMINATION OF ENERGY

Energy was computed as follows

Calculation-

Energy(kcal)=(protein(g)×4)+(carbohydrate(g) ×4+(fat(g) ×9)

APPENDIX- XI

DETERMINATION OF CALCIUM

METHOD – Complexometric titration

REAGENTS – HCl, HNO 3, methyl red., NH 4 OH, ammonium oxalate, H2So4,0.02M KMnO 4

PROCEDURE:

- Weigh accurately about 2g of sample in a porcelain dish.
- Ignite in furnace to carbon free ash, but avoid fusing.
- Boil the residue in 40ml HCl (1+3) and few drops of HNO 3.
- Cool and transfer to a 250ml standard flask, dilute to volume and mix.
- Pipette 25ml clear liquid into a beaker, dilute to 100ml and add 2drops of methyl red.
- Add NH 4 OH (1+1) drop wise to pH 5.6 (brownish orange color).
- If overstepped add HCl (1+3) with dropper to orange.
- Add two more drops of HCl to pink and pH 2.5-3.0
- Dilute to 150ml and boil.
- Add slowly with constant stirring 10ml of hot saturated (4.2%) solution of ammonium oxalate.
- If red changes to orange or yellow, add HCl drop wise until pink
- Let stand overnight for precipitate to settle.
- Filter the supernatant through Whatman no.40 and wash the precipitate thoroughly with NH 4 OH (1+50).
- Place the paper in original beaker and add a mixture of 125ml water and 5ml H 2 SO 4 .
- Heat to 70˚C and titrate against 0.02M KMnO 4 (0.1N) to slight pink Color.

CALCULATION:

Calcium (as Ca) = Titre volume× normality of KMnO 4 ×100×28×40 / Sample weight×1000×56

APPENDIX-XII

DETERMINATION OF IRON

METHOD - Atomic absorption method

APPARATUS:

Atomic absorption spectrophotometer with air acetylene flame Cathode Lamp-Fe – 248.3 nm.

REAGENT:

- Fe (NIST traceable)
- Nitric acid (1:499).
- CaCl 2 solution:

Dissolved 630 mg CaCO 3 , 50 ml of 20% v/v HCL, if required boil gently to obtained complete solution. Cool and dilute to 1000 ml with distilled water.

PROCEDURE:

- Take 100 ml standard flask
- Prepare Iron standards (Nist traceable) to 0.05,0.1, 0.125,0.15,0.20&0.25 mg/l
- in nitric acid (1:499) from 1000 ppm solution.
- Prepare a blank solution in 100ml distilled water.
- Pipette out 100 ml of sample in a beaker and digest with 0.5 ml. of conc. Nitric acid and add 25 ml CaCl 2 till the volume reduced to three fourth.
- Make up to 100 ml. with distilled water.
- Process the blank also in the above manner.
- Set the AAS as per the specific work instruction.
- Aspirate the blank, standards and DIGESTED FOOD SAMPLE solutions.
- Measure the absorbance of the iron at 248.3nm.

CALCULATION:

Draw the standard calibration graph by plotting the absorbance Vs standard conc. for each standard

Process one quality check standard at 0.05 mg/l along with each batch of samples.

APPENDIX-XIII

DETERMINATION OF TOTAL DIETARY FIBER

METHOD – Proxy method

REAGENTS - Petroleum ether, phosphate buffer, 0.3N NaOH, α-amylase, 0.3N HCl

PROCEDURE:

Blank was run along with samples to measure any contribution from reagents to residue. Defatting of samples have done with 25ml of petroleum ether / g of sample three times to remove fixed oil. Then, Weigh 1gm of sample. Phosphate buffer (pH 6.0, 50 mL) was added to sample. Adjust to pH 6.0 ± 0.2 by adding 0.3N NaOH or 0.3N HCl. Enzyme hydrolysis of sample was started by adding 0.1mL α-amylase, incubate at 95 – 100 °C for 30 minutes in water bath with continuous agitation. Cool to room temperature. Adjust to pH 7.5 ± 0.2 by adding 10ml of 0.3N NaOH. Papain 5mg was added, incubate at 60 °C for 30 minutes in water bath with continuous agitation. Cool to room temperature. The pH was adjusted to 4.0 – 4.6 by adding 10ml 0.3N HCl. Amyloglucosidase 0.3mL was added, incubate at 60 °C for 30 minutes let precipitates to form and filter it. Weigh the residue.

Estimate soluble dietary fibers, filtrates plus washing were mixed with 400mL of 95% ethanol to precipitate materials that were soluble in the digestates. After 1h, precipitates were filtered. Residue was washed successively three times with 20mL of 78% ethanol and two times with 10mL of 95% ethanol and then acetone respectively.

For insoluble dietary fiber estimation, residue was washed with 10mL of water (for removing soluble dietary fibers), 95% ethanol and then acetone respectively. Residue was dried at 105°C for 5h in hot air oven, cool in dessicator and weigh to 0.1mg separately (S1 and S2).

The soluble and insoluble dietary fibers (%) were calculated by using following formula. Sol.DF % = (Residue (S1)- Protein-Ash – Blank) X 100
Weight of test portion

Insol.DF % = (Residue (S2)- Protein-Ash – Blank) X 100 Weight of test portion

APPENDIX -XIV

DETERMINATION OF FLAVONOIDS

PRINCIPLE:

The principle of this method is that flavonoids react with aluminium chloride to form a stable complex with a maximum absorbance at 510 nm. The TFC is determined by measuring the absorbance of the complex formed and comparing it with the absorbance of a standard solution of quercetin.

REAGENT:

- Distilled water
- 5% aqueous sodium nitrite solution
- 10% aqueous aluminium chloride solution and
- 4% aqueous sodium hydroxide solution
- Quercetin is used as the standard

PROCEDURE:

- Dilute the ethanolic extracts of sample with distilled water and adding 5% aqueous sodium nitrite solution, followed by 10% aqueous aluminium chloride solution.
- After 5 minutes, 4% aqueous sodium hydroxide solution is added, and the volume is made up with distilled water.
- The absorbance of the mixture is measured at 510 nm using a UV spectrophotometer.
- The TFC is quantified using a standard curve prepared with quercetin at concentrations ranging from 0.2 to 1.0 mg/mL in 70% methanol.
- Repeat the experiment at least three times to ensure accuracy and precision of the results.

CALCULATION:

The TFC is calculated as follows:

$$TFC\ (mg\ QE/g\ dw) = (A \times B \times C) / D$$

Where,

A is the absorbance of the sample

B is the dilution factor

C is the volume of the sample (in mL)

D is the dry weight of the sample (in g). The results are expressed as mg quercetin equivalent per gram of dry weight (QE g^{-1} dw)

APPENDIX-XV

DETERMINATION OF CAROTENOID

CALCULATION

For total carotenoids

Amount of total carotenoids present= $\frac{P \times 4 \times V \times 100}{W}$ mg

Where ,

P- Optical density of the sample V- Volume of thesample

W- Weight of thesample

For Lycopene

Mg of lycopene per 100g of sample=

$$\frac{3.1206 \times OD \times Volmade\ up \times dilution \times 100}{1 \times Weight\ of\ the\ sample \times 1000.}$$

APPENDIX-XVI

DETERMINATION OF TOTAL PHENOLS

PRINCIPLE:

Phenols react with phosphomolybdic acid present in Folin Ciocalteau reagent in alkaline medium to produce a blue colored compound, which is read colorimetrically.

REAGENTS:

- 70% ethanol
- Folin Ciocalteau reagent
- 20% Sodium Carbonate
- Stock standard Gallic acid-5 mg of Gallic acid dissolved in 100 ml distilled water
- (50 µg/ml)
- Working standard Gallic acid-1 in 10 dilution - Conc. 5 µg/ml

PROCEDURE:

- Weight exactly 5 g of the sample and extract it with 70% ethanol.
- Centrifuged the homogenate at 1500 rpm for 20 mins and the supernatant is saved.
- Aliquots of the standard are taken in separate test tubes from 0.2 to 1 ml with concentration of 1 to 5 µg/ ml respectively.
- 0.5 ml of the sample is taken in duplicates.
- The solutions are made up to 1 ml with the distilled water.
- Add 1.5 ml of Folin- Ciocalteau reagent to the test tubes and kept in the room temperature for 4 mins.
- After 4 minutes add 1.5 ml of 20% sodium carbonate to each of the test tubes.
- Mix the solutions thoroughly and the absorbance was measured in the colorimeter at 660 nm against the reagent blank.

CALCULATION:

The values are plotted in the graph. From the graph the concentration of phenols in the sample is estimated and expressed as mg of gallic acid per gram of 100g of fruit powder.

APPENDIX-XVII
DETERMINATION OF TOTAL SUGARS

PRINCIPLE

Total reducing sugars represent reducing sugars and non-reducing di and oligo saccharides,which can be hydrolysed into reducing sugars with dilute acids.

REQUIREMENTS

Equipment and Apparatus-

Chemical balance, 1mg sensitivity Hot plate Burette (50 ml cap.) with an off-set tip Volumetric flask, 250 ml Pipette, 5 ml and 25 ml Conical flask, 250 ml Weighing bottle Funnel (small) Whatman No. 1 filter circles .

Chemicals and Reagents

- Fehling's solution A: Dissolve 69.28 g copper sulphate (CuSO4.5H2O) in distilled water and dilute to 1000 ml. Filter and store in amber colour bottle.
- Fehling's solution B: Dissolve 346 g Rochelle salt (Potassium sodium tartrate: KNa C4H4O6. 4H2 O) and 100 g NaOH in distilled water. Dilute to 1000 ml. Filter and store in amber colour bottle.
- Neutral lead acetate solution: Prepare 20% neutral lead acetate solution. Potassium oxalate solution: Prepare 10% potassium oxalate (K2C2O4. H2O) solution.
- Methylene blue indicator: Prepare 1% methylene blue solution in distilled water.

PROCEDURE

Pipette an aliquot of 50 ml of the clarified, de-leaded filtrate to a 100 ml volumetric flask. Add 5 ml of conc. HCl and allow to stand at room temperature for 24 hours. Neutralise with conc. NaOH solution followed by 0.1N NaOH using phenolphthalein as end point indicator. Make up to volume and transfer to 50 ml burette having an off-set tip. Perform the titration against Fehling's solution similar to the procedure described for reducing sugars, and determine the total sugars as invert sugars.

OBSERVATIONS

Volume of the acid hydrolysed sample solution required for Fehling solution (titre) =

V4 = ------ ml

CALCULATIONS

Based on the factor for Fehling's solution, total reducing sugars in

V4 ml = 0.0025 × V1 g 21

As 50 ml of the clarified and de-leaded solution is diluted twice (50 ml to 100 ml) after hydrolysis, dilution volume of the sample = (2 × V2).

$$\text{Therefore, \% Total reducing sugars} = \frac{0.0025 \times V1 \times 2 \times V2 \times 100}{V4 \times W}$$

$$= \frac{0.5 \times V1 \times V2}{V4 \times W} = Y\ \%$$

Total reducing sugars comprises of reducing sugars and non-reducing sugars, which can be hydrolysed into reducing sugars under the experimental conditions.

This non-reducing sugar is usually expressed in terms of sucrose.

As 0.95 g sucrose on hydrolysis yields 1 g invert sugar (glucose + fructose):

% Sucrose in the sample = (Total reducing sugars − % Reducing sugars originally present) × 0.95 = (Y – X) × 0.95 [% Total sugars = (% Reducing sugars + % Sucrose)]

RESULTS

Sucrose content in the sample = % by weight

APPENDIX -XVIII

DETERMINATION OF GLUTEN

PROCEDURE

Weigh accurately into a dish about 25 g of the material. Add abou't 15 ml of water to the material and make it into a dough, taking care to see that all the material is taken into the dough~ Keep the dough gently in a beaker filled with water and let it stand for one hour. Remove the dough and place it in a piece of bolting silk cloth with an aperture of 0·16 rom size (No. 10 XXX) and wash it with a gentle stream of tap water. water passing through the silk does not turn blue when a drop of iodine solution is added to it. Spread the k tight on a porcelain plate for facilitating scraping. Transfer the residue from the silk by means of a spatula to a tared porcelain dish. Spread the wet gluten into a thin layer and cut into small pieces. transfer any residue sticking to the spatula into the porcelain dish. Place the porcelain dish in an air-oven maintained at 133 ± 2°0. Dry for two hours. Cool in a desiccator and weigh.

CALCULATION

$$\text{Gluten (on dry basis), percent Mass} = \frac{10\,000\,(W2 - WI)}{W\,(100 - M)}$$

where

W2 - mass in g of porcelain dish with dry gluten,

W1 -mass in g of the empty porcelain dish,

W - mass in g of the material taken for the test, and

M - moisture, percent by mass

APPENDIX-XIX

NUTRIENT AND NUTRACEUTICAL ANALYSIS OF STANDARD UNIBIC COOKIES

ENVIRO FARMERS LABS & TECHNOLOGIES

SERVICES :
- Water and Waste Water Testing
- Environmental Monitoring & Testing
- Testing of Food and Food Products
- Soil, Compost & Solid Waste Testing
- Microbial Testing Services
- Other Testing Services

CONTACT US :
Address : No-2/83, Avinashi Road Main Road, Chinniampalayam Post, Sulur Taluk, Coimbatore - 641062, Tamilnadu, India.
Phone : + 91 9042855456 + 91 7530035465
+ 91 9952855456 + 91 7530035470
E-Mail : envirofarmerslabtech@gmail.com
Website : www.efltcbe.com

ACCREDITATIONS :
ISO 9001 : 2015 CERTIFIED
FAO Food and Agriculture Organization

TEST REPORT FOR SAMPLE ANALYSIS			
Report Code	EFLT/TRE/2023/2479	Report Date	03.04.2023

CUSTOMER DETAILS

Name & Address of the Customer/Company	M/s. AARTHI SWETHA.M, M.Sc. Department of Nutrition & Dietetics, Avinashilingam university, Coimbatore , Tamilnadu, India.

DETAILS OF SAMPLE

Nature of Sample	Standard Cookies	Sample Quantity	223 g
Customer Code	Standard Cookies	Laboratory Code	EFLT/FA/2023/2479
Sample Collected On	31.03.2023	Sample Collected By	Customer
Sample Received On	31.03.2023	Analysis Started Date	31.03.2023
Received Condition	Good	Analysis Completed Date	03.04.2023

TEST REPORT PARTICULARS

S.No	Parameters	Test Method	Unit	Result
1.	Moisture	IS 12711:1989 RA 2015	g/100 g	6.23
2.	Total ash	IS 12711:1989 RA 2015	g/100 g	4.92
3.	Fat	IS 12711:1989 RA 2015	g/100 g	19.2
4.	Total protein	IS 7219:1973 RA 2020	g/100 g	5.23
5.	Carbohydrate	IS 1656:2007 RA 2019	g/100 g	64.42
6.	Energy	EFLT/SOP/2019	Kcal/100 g	453.2
7.	Calcium as Ca	IS 5949	mg/100 g	46.3
8.	Iron as Fe	IS 12711:1989 RA 2015	mg/100 g	4.26
9.	Crude Fibre	AOAC 20th Edn 944.13	g/100g	6.22
10.	Flavonoids	FSSAI Manual for Additives	mg/100g	1.23
11.	Carotenoid	FSSAI Manual for Additives	mg/100g	0.36
12.	Phenol	FSSAI Manual for Additives	mg/100g	0.02
13.	Total sugars	FSSAI Manual for Additives	g/100g	39.2
14	Gluten	IS 1155	g/100g	7.23

End of Report

Authorized Signatory
Technical Manager
S.P.Mohanraj

ENVIRO FARMERS LABS & TECHNOLOGIES COIMBATORE TAMILNADU INDIA

APPENDIX-XX

NUTRIENT AND NUTRACEUTICAL ANALYSIS OF GLUTEN-FREE COOKIE (VARIATION-IV)

ENVIRO FARMERS LABS & TECHNOLOGIES

EFLT

SERVICES :
- Water and Waste Water Testing
- Environmental Monitoring & Testing
- Testing of Food and Food Products
- Soil, Compost & Solid Waste Testing
- Microbial Testing Services
- Other Testing Services

CONTACT US :
Address : No-2/83, Avinashi Road Main Road, Chinniampalayam Post, Sulur Taluk, Coimbatore - 641062, Tamilnadu, India.
Phone : + 91 9042855456 + 91 7530035465 + 91 9952855456 + 91 7530035470
E-Mail : envirofarmerslabtech@gmail.com
Website : www.efltcbe.com

ACCREDITATIONS :
ISO 9001 : 2015 CERTIFIED
FAO FIAT PANIS Food and Agriculture Organization

TEST REPORT FOR SAMPLE ANALYSIS			
Report Code	EFLT/TRE/2023/2479A	Report Date	03.04.2023
CUSTOMER DETAILS			
Name & Address of the Customer/Company		M/s. AARTHI SWETHA.M, M.Sc. Department of Nutrition & Dietetics, Avinashilingam university, Coimbatore , Tamilnadu, India.	
DETAILS OF SAMPLE			
Nature of Sample	Cookies	Sample Quantity	231 g
Customer Code	Gluten free Cookies	Laboratory Code	EFLT/FA/2023/2479A
Sample Collected On	31.03.2023	Sample Collected By	Customer
Sample Received On	31.03.2023	Analysis Started Date	31.03.2023
Received Condition	Good	Analysis Completed Date	03.04.2023

TEST REPORT PARTICULARS

S.No	Parameters	Test Method	Unit	Result
1.	Moisture	IS 12711:1989 RA 2015	g/100 g	5.23
2.	Total ash	IS 12711:1989 RA 2015	g/100 g	5.02
3.	Fat	IS 12711:1989 RA 2015	g/100 g	16.1
4.	Total protein	IS 7219:1973 RA 2020	g/100 g	6.23
5.	Carbohydrate	IS 1656:2007 RA 2019	g/100 g	67.42
6.	Energy	EFLT/SOP/2019	Kcal/100 g	442.1
7.	Calcium as Ca	IS 5949	mg/100 g	41.6
8.	Iron as Fe	IS 12711:1989 RA 2015	mg/100 g	6.23
9.	Crude Fibre	AOAC 20th Edn 944.13	g/100g	7.11
10.	Flavonoids	FSSAI Manual for Additives	mg/100g	1.03
11.	Carotenoid	FSSAI Manual for Additives	mg/100g	0.62
12.	Phenol	FSSAI Manual for Additives	mg/100g	0.13
13.	Total sugars	FSSAI Manual for Additives	g/100g	38.1
14	Gluten	IS 1155	g/100g	3.14

****End of Report****

Authorized Signatory

Technical Manager
S.P.Mohanraj

ENVIRO FARMERS LABS & TECHNOLOGIES COIMBATORE TAMILNADU INDIA EFLT