WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.453
Volume 14, Issue 5, 563-612. Research Article ISSN 2277–7105

DIETARY SUPPLEMENTS AND NUTRACEUTICALS

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Article Received on
06 January 2025,

Revised on 26 Jan. 2025,
Accepted on 16 Feb. 2025

DOI: 10.20959/wjpr20255-34914

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INTRODUCTION

Definition: A dietary supplement is a product taken by mouth that contains a "dietary ingredient" intended to supplement the diet.

E.g. - Vitamins, Minerals, Amino acids etc…

"A Nutraceutical is any substance that is a food or a part of food in provides medical or health benefits, including the prevention and treatment of disease".

E.g.-Selenium, Lycopene.

Nowadays, diet is thought to be much richer than it used to be. The people's ignorance of the basic principles of nutrition has led a large part of the population to a non-balanced diet that is high in both calories and fat and low in proteins, vitamins and minerals. This long-term situation has led to the emergence of various degenerative diseases. In an effort to address this concern, nutritional supplements was proposed to proper solution to this problem.

Dietary supplements are products that contain one or more concentrated nutrients with the aim of supplementing an individual's daily diet, when his or her diet is not balanced or if nutrients are lacking. Dietary supplements do not belong to the category of common food neither medicines nor special dietary products and not intended for specific categories of people. Supplements offer the missing ingredients to the body in order for the latter to be kept in good physical and mental condition. In effect, the human system wouldn't be exhausted and at the same time injuries and fatigue are avoided.

The production as well as the consumption of dietary supplements have considerably increased in the last decade. Majority of these supplements are supplied in the form of tablets or powder. Although, the increased intake is supposed to offer health benefits, too much consumption may result in higher amounts of vitamins and minerals which the body may not

be able to tolerate. As a result, consumers are exposed to health risks due to excessive consumption of dietary supplements. The problem becomes more serious if people take these supplements by themselves, without prescription or medical supervision.

Today's dietary guidelines from health and nutrition agencies all over the world cover more than 40 nutrients that are subdivided into 6 categories:

1. Carbohydrates
2. Fats
3. Proteins
4. Vitamins
5. Minerals
6. Water

Daily nutrient recommendations are collectively known as dietary reference intakes (DRIs). A healthy diet is one that favours "real" fresh whole foods that have been sustaining people throughout the millennia. Whole foods supply the needed vitamins, minerals, protein, carbohydrates, fats, and fibre that are essential to good health.

A balanced diet is a mix of food from the different food groups

1. Vegetables
2. Legumes
3. Fruits
4. Grains
5. Protein foods
6. Meat
7. Dairy

Variety involves eating different foods from all the food groups that helps to ensure that you receive all the nutrients necessary for a healthy diet. The components of the Mediterranean diet have been evaluated as substantially beneficial to human health.

The World Health Organization (WHO) makes the following recommendations for a balanced and healthy diet:

(a) Eat roughly the same amount of calories that your body is using.

(b) A healthy weight is a balance between energy consumed and energy that is 'burnt off'.

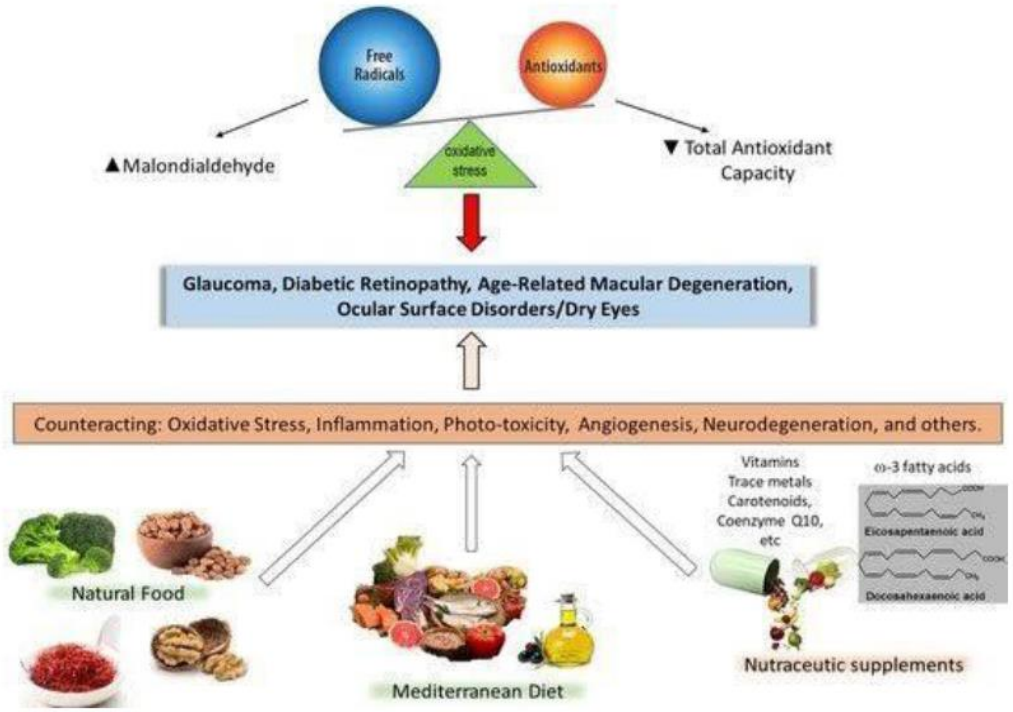
(c) Limit intake of fats, and prefer unsaturated, than saturated fats and trans fats.

(d) Increase consumption of plant foods, particularly fruits, vegetables, legumes, whole grains and nuts.

(e) Limit the intake of sugar, salt/sodium consumption from all sources and ensure that salt is iodized.

(f) Eat a diet with essential micronutrients such as vitamins and certain minerals.

In contrast, many commercially prepared foods or fast foods as they are called, often lack nutrients and contain inordinate amounts of sugar, salt, saturated and trans-fats, all of which are associated with the development of diseases.



Some classes of supplements, Their Examples and Contents are presented in Table.

Class	Example	Contents
Activator	Amino acids	Contains growth hormone and other hormones
Carbohydrate	Dextrose	May contain vitamins and electrolytes
Food and Food stuff	Fish oils, garlic, royal jelly, yeast	Contain mineral and vitamins
Herbs	Ginseng, Fiber	Contains amino acids, other plant source
Minerals	Selenium, multimineral tablets	Contains only minerals

Multivitamins and multiminerals	Vitamin D, calcium supplement	Contains both mineral and vitamins
Oil supplements	Cod liver oil, primrose oil	Contains oil base, with vitamins, minerals
Vitamins	Vitamin C, vitamin B	Contains only vitamins

1. Vitamins and Minerals as Supplements.
2. Fish Oil and Omega-3 Fatty Acids as Supplements.
3. Protein Powders and Infant Formula as Supplements.
4. Botanical Supplements (Plant based).

Introduction of gluten in wheat barn

Wheat is a valuable source of essential nutrients, providing carbohydrate-based energy and fibre, protein, B vitamins, calcium, magnesium, phosphorus, potassium, zinc, and iron. In low and medium-income countries, grain-based foods still make up the central part of the diet.

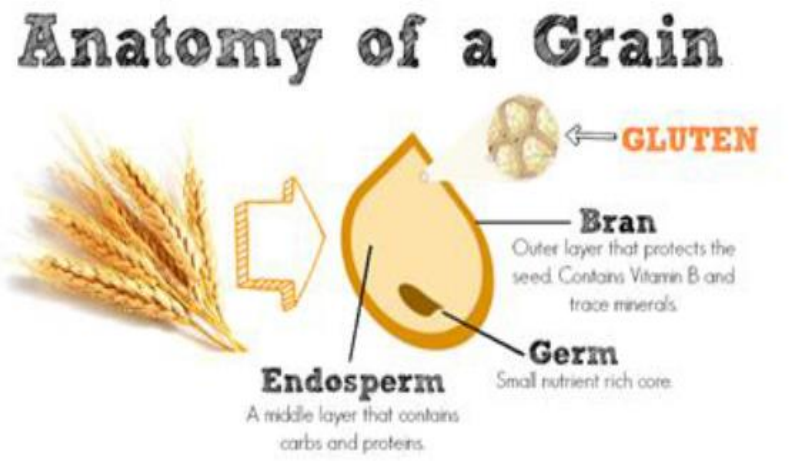
The wheat seed can be ground into flour or semolina, for example, which form the essential ingredients of bread, pasta, noodles, and other food products, essentially the primary source of nutrients for most of the world population. Conversely, the lack of grains too often signifies hunger and malnutrition. The characteristic that has given wheat an advantage over other temperate crops is the unique viscoelastic properties of dough formed from wheat flours, which allow it to be processed into such an array of forms**. Dough viscoelasticity depends on the structures and interactions that occur between grain storage proteins that form the gluten protein complex.**

Gluten, which is now an almost ubiquitous ingredient in the food industry, is implicated in several immune-mediated disorders, such as celiac disease (CD). Both CD and other intolerances are of increasing concern], and the prevalence of CD is predicted to rise. These disorders demand a gluten-free diet (GFD), but a GFD can itself be associated with digestive problems due to insufficient intake of dietary fiber and other nutrients.

This review focuses on wheat from a human health perspective. We will present the positive impacts of wheat, referring to the benefits of the different components of the wheat grain on human health, and adjacent to this with the negative impacts on the health of sensitive and genetically susceptible individuals caused by wheat components. At the same time, we draw attention to common gluten-related misconceptions and try to demystify them.

The health benefits of wheat

Wheat grain is composed of the germ (2–3%), the bran (13–17%), and the endosperm (80–85%). Wheat germ is the embryo of the wheat kernel and is relatively rich in protein, lipids, and several of the B-vitamins. Whole-wheat flour includes the bran, which contains a limited amount of protein, larger quantities of the B-complex vitamins, trace minerals, and indigestible cellulose material called dietary fiber. White flour originates from the endosperm. The endosperm contains most of the protein in the whole kernel, iron, carbohydrates, and many B-complex vitamins, such as riboflavin, thiamine, and niacin.



The consumption of wheat brings many health benefits. In the European Prospective Investigation into Cancer and Nutrition (EPIC) study populations, 27% of total carbohydrate intake was from bread. Epidemiological studies show that cereal dietary fiber and wholegrain consumption protects against the fast increasing chronic diseases related to a sedentary lifestyle, such as type 2 diabetes and cardiovascular disease.

1. Proteins

Protein is an essential nutrient for humans and animals. Protein content is used to classify wheat. Breeders target this feature by regularly selecting for protein content traits in breeding programs; wheat with a low protein content is suitable for animal feed other uses, while wheat with a high protein content is necessary for bread making. Protein content differs depending on the growing conditions, type or class of wheat, and fertilizer Foods of 24 inputs, especially nitrogen. Thus, there is no such thing as typical protein content, but on average, it can vary between 9–18% of the grain weight. Protein is unequally distributed in

the grain. A percentage of 5.1% of protein was reported in the pericarp, 5.7% in the testa, 22.8% in the aleurone, and 34.1% in the germ. T.B. Osborne demonstrated that wheat proteins could be classified according to their extractability and solubility in distinct solvents. Globulins are insoluble in pure water, and high NaCl concentrations but soluble in dilute NaCl solutions; albumins are soluble in water; glutenins are soluble in dilute acid or sodium hydroxide solutions, and gliadins are soluble in 70% ethyl alcohol. Of the 20 amino acids commonly present in proteins, lysine, leucine, isoleucine, phenylalanine, threonine, tyrosine, tryptophan, histidine, valine, and methionine (and potentially cysteine since it can only be synthesized from methionine) are considered essential because they must be provided in the diet as animals cannot synthesize them.

2. Carbohydrates

The wheat grain consists of 85% carbohydrate at maturity, 80% of which is the starchy endosperm. The non-starch carbohydrate is constituted of approximately 7% mono-, di-, and oligosaccharides and fructans, along with about 12% of cell wall polysaccharides]. In addition to being an essential energy source in the human nutrition and animal feed, wheat starch is the substrate for the production of alcoholic beverages and fuel ethanol by fermentation and is the raw material for several other industries. Polysaccharides are the main structural elements of the protoplasts walls present in all cells of the grain tissues. The cell wall polysaccharides are essential in human diet as sources of dietary fiber and have an impact on end-use quality and grain consumption.

3. Lipids

Lipids are a minor constituent of wheat, mostly in the germ, making up 3–4% of the whole grain weight and 1–2.5% of directly milled flour. Lipids have a critical role in baking processes, dough mixing, and the acceptance of the finished products by consumers. Their ability to associate with gluten proteins and form complexes contributes to stabilizing the gas-cell structure, significantly influencing the final texture of baked products and loaf volume.

4. Minerals

Iron is concentrated in the aleurone and zinc in the embryo. Deficiencies in iron and zinc micronutrients are common in populations that consume wheat as a staple because wheat products are usually low in bioavailable forms of these micronutrients. In wheat, two features contribute largely to the low content in bioavailable iron and zinc: the most consumed form is

white flour, which contains low concentrations of these minerals, and the existence of phytates in mineral-rich bran fractions that retain minerals in a form that is not bioavailable.

5. B. Vitamins

The B vitamin complex, which at first was thought to be a single compound, comprises eight water-soluble components, which often co-occur in the same foods. They are unequally spread in the wheat kernel and are primarily found in wheat bran and the germ; hence they are present in reduced quantities in refined flours. Cereals are dietary sources of several B vitamins, particularly riboflavin (B2), folates (B9), thiamine (B1), pyridoxine (B6), and niacin (B3). These molecules play an essential role in metabolism, particularly thiamine in the metabolism of carbohydrates, and riboflavin and pyridoxine in the metabolism of proteins and fats. Consumption of wholemeal products provides 40% of the recommended daily allowance of thiamine, 10% of riboflavin, 22% of niacin, 33% of vitamin B6, and 13% of folate recommended. Niacin is of particular concern as only a proportion of the total present in cereals is bioavailable in a chemically bound form, nicotinic acid. Multiple studies have focused on this issue, with some reports of increasing niacin bioavailability by treatment with alkali.

5. Phytochemicals

Two main groups of phytochemicals, derived from different biosynthetic pathways, are present in wheat grain: phenolics and terpenoids. The primary group of phytochemicals in wheat grain are phenolic acids, but numerous other phenolic compounds have been identified, including lignans, alkylresorcinols, and flavonoid. Phenolic compounds are characterized by at least one aromatic ring carrying at least one hydroxyl group. Phenolic acids have vigorous antioxidant activity, and the total phenolic content is correlated with total antioxidant activity.

The importance of antioxidant properties for human health is widely discussed; however, evidence that phenolic compounds, including ferulic acid, the major phenolic acid in wheat, improve vascular function in humans is increasing.

6. Wholegrain

Since the 1900s, when Dr. Thomas Allinson promoted Allinson's bread as a healthier lifestyle, the intake of wholegrain wheat has been promoted for its health benefits. The interest in studying the wholegrain wheat composition has been increasing in order to identify compounds that promote health and better recognize the full potential of wheat in disease

avoidance and health. Wholegrain wheat products include various components with recognized or proposed health benefits, including dietary fiber, phenolic acids, carotenoids, flavonoids, sterols, lignans, selenium, magnesium, alkylresorcinols, tocopherols, and B-complex vitamins, which are mainly presented in the bran. Consequently, they are either absent or present in lower amounts in white flour, which is almost exclusively derived from endosperm starch cells. While other whole grains may contain similar components, wheat eminence in the diet potentially makes this cereal a more significant contributor to the intake of these compounds.

Wholegrain wheat may protect against the development of diseases related to chronic diet. Extensive cohort studies have described a noticeably reduced risk of cardiovascular disease type 2 diabetes, and certain forms of cancer.

Wheat/Gluten-Related disorders

Wheat/gluten-related diseases can be classified into three different disorders: autoimmune, allergic, and neither autoimmune nor allergic. Celiac disease is the most prominent autoimmune gluten-related disorder (CD). It is a condition of the small intestine caused by gluten and gluten-related proteins and influenced by environmental and genetic factors. An IgE and non-IgE mediated immune response characterize wheat allergy (WA), resulting in an allergic reaction in some individuals upon contact, inhalation, or uptake of foods containing wheat but not necessarily other grains as barley or rye. However, IgE-cross reactivity to other cereals is possible in some people. Patients with non-celiac wheat/gluten sensitivity (NCWGS) experience identical symptoms to CD, but they do not test positive for CD.

Celiac disease

The binding of gluten peptides to T cells triggers CD in some individuals expressing human leukocyte antigen (HLA) DQ2 or DQ8 in cells specialized in presenting antigens. Specific CD4+ T cells then recognize the presented peptides releasing inflammatory cytokines, leading to changes in the architecture of intestinal mucosa with atrophy and flattening of villi that can lead to total villous degeneration and enteropathy. Moreover, gliadin peptides are responsible for the activation of innate immunity of the intestinal epithelial cells. Hence, the gliadin peptides can directly stimulate the immune response of macrophages and dendritic cells through pattern recognition receptors (PRR), such as toll-like receptors (TLRs). It has also been demonstrated that tissue transglutaminase, an enzyme involved in the deamidation of glutamine residues to glutamate, present in the intestinal epithelium, plays an important

role by increasing the binding affinity of gluten peptides to HLA-DQ2 and DQ8 heterodimeric receptors.

The expression of the major histocompatibility complex (MHC) class II molecules is related to genetic risk factors. HLA-DQ2 and HLA-DQ8 are the most potent genetic risk factor in CD since they are critical in initiating detrimental immune responses. Nevertheless, additional genetic variations are reported as risk factors in CD, which means HLA-DQ2 and HLA-DQ8 do not account for all the genetic susceptibility to CD. For example, two cytokines implicated in CD pathogenesis are encoded by specific gene polymorphisms of IL-2/IL-21. In the past, non-HLA genetic risk factors were reported. The Foods consumption of gluten and gluten-related proteins is the main environmental risk factor for CD, which employs a strong immunodominant function in people with a genetic susceptibility to CD.

Studies of the timing of gluten introduction in infants' diets suggest that infants who began to receive gluten either before four months or after seven months of age have more risk of developing CD. This conclusion supports the notion that there is a time-space between four and seven months of age during which the introduction of gluten might induce the tolerance to CD. However, more recent studies refute this statement, showing no evidence that avoidance of either early (at four months of age) or late (at/after six or even twelve months) gluten introduction puts children at risk of CD. Environmental factors can also play a pathogenetic role in the disease. Studies conducted on twins showed that, in 25% of the cases, one of the two twins did not develop CD, supporting this environmental hypothesis.

Non-Celiac Wheat/Gluten Sensitivity

Non-celiac wheat/gluten sensitivity (NCWGS) makes people experience symptoms similar to CD and WA. However, patients with NCWGS do not have specific IgE against wheat proteins or IgA anti-TG2 autoantibodies. The symptoms develop in a few hours or days after wheat/gluten consumption and include abdominal distension, abdominal pain, diarrhea, gas, among others. Patients also experience extraintestinal symptoms, including headache, fatigue, pain in muscles and joints, and eczema. Recent studies have given rise to the idea that other wheat components, such as oligosaccharides like fructans, 15 Foods 2021, α-amylase/trypsin inhibitors, and wheat-germ agglutinin, may contribute to the development of NCWGS. The pathogenic mechanisms of NCWGS are far from understood.

Preliminary data indicate that activation of innate immunity triggers NCWGS without the

involvement of adaptive immunity, which would be a crucial factor in CD development. The increased expression of toll-like-receptors (TLRs), a protein class that plays a vital role in innate immunity, in the small intestine is the evidence supporting the hypothesis of the activation of innate immunity in NCWGS. TLR2, TLR1, and TLR4 have been identified in the intestinal mucosa and some cells of the lamina propria of patients with NCWGS. There is diverging information on intestinal permeability in NCWGS. A study conducted in 2011 determined the gut permeability of NCWGS and CD patients using the urine lactulose/mannitol test.

The small intestines of NCWGS patients were significantly less permeable than those of CD patients and controls. Moreover, duodenal biopsies of NCWGS patients found higher expression of claudin-4 mRNA, a marker of reduced permeability. By comparison, another study reported a subgroup of HLADQ2/DQ8+ patients with diarrhea-predominant irritable bowel syndrome following a gluten challenge that had increased intestinal permeability.

Moreover, Hollonetal (2015), in an ex vivo study, evaluated alterations in transepithelial electrical resistance (TEER) of tissue biopsies from NCWGS patients, active CD patients, CD patients in remission and controls subjected to pepsin-trypsin digested gliadin. This study has shown that exposure to gliadin increases intestinal permeability and decreases TEER in all patient groups compared to controls. This discrepancy suggests that further studies are required to define the small intestine's permeability in NCWGS and improve our overall knowledge about it.

Other Autoimmune Wheat/Gluten-Related Diseases

Gluten ataxia

Gluten ataxia (GA) is a form of cerebellar ataxia, affecting mainly Purkinje cells, and is caused by antibodies released when digesting gluten that mistakenly attacks part of the brain in individuals that are sensitive and genetically susceptible. The clinical symptoms of GA are identical to those of other ataxias. They include gait ataxia (100%), lower limb ataxia (90%), gaze-evoked nystagmus (84%), upper limb ataxia (75%), ocular signs like dysarthria (66%), and other movement disorders including chorea, myoclonus, opsoclonus myoclonus, and palatal tremor.

Dermatitis herpetiformis

Dermatitis herpetiformis (DH), repeatedly associated with CD, is an autoimmune, chronic,

and recurrent cutaneous-intestinal disorder detected in genetically susceptible individual. Anti-tTG antibodies that also recognize epidermal transglutaminase (ETG) can be produced after exposure to gluten. ETG is homologous to tTG in terms of structure and is the primary antigen in DH. IgA antibody deposition in dermal papillae causes pruritic, vesiculobullous, and localized lesions in DH patients. DH affects the extensor surfaces such as knees, buttocks, elbows, and scapular areas.

Wheat allergy

Allergens cause allergic reactions, and wheat is one of the five most frequent foods causing them in children. After milk and eggs, wheat is the most common allergen in Japan, Germany, and Finland. In children and adults, wheat allergy (WA) prevalence is approximately 1% depending on age and region. In contrast to CD, distinct wheat components such as water-insoluble proteins (gliadin and glutenin) and water/salinesoluble proteins (albumin and globulin) contribute to the development of WA.

IgE-Mediated Wheat Allergy

IgE-mediated WA is triggered by allergen ingestion (food allergy), inhalation (respiratory allergy), or skin contact (dermal allergy). The antigen is introduced by dendritic cells that trigger CD4+ T cells to differentiate into T helper type 2 (Th2) cells. These cells produce cytokines such as IL-4, IL-5, and IL-13 that stimulate B cells to produce IgE. When a new exposure to wheat allergens occurs, the IgE antibodies that are bound to their high-affinity receptor (FcεRI) on basophils or mast cells, recognize specific epitopes in wheat allergens.

The recognition results in IgE-crosslinking that triggers the release of vasoactive mediators like histamine from mast cells or basophils, leading to allergic responses, including WA. The most common symptoms of WA due to these mechanisms include gastrointestinal symptoms (nausea, abdominal pain, vomiting, diarrhea), dermal (itching, eczema, redness), respiratory (rhinitis, asthma), circulatory (flushing, angioedema), and cerebral (disturbed thinking, headache, dizziness) which typically manifest minutes to hours after exposure.

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a particular type of IgEmediated WA. This condition gives rise to severe anaphylactic reactions to wheat when intense exercise is practiced soon after being consumed. Symptoms of WDEIA include angioedema, chest pain, diarrhea, dysphagia, dyspnea, flushing, headache, hoarseness, nausea, pruritus, and syncope.

Baker's asthma is also an IgE-mediated WA that develops after allergen inhalation, especially cereal flour dust present in the work environment, and affects 0.03–0.24% of pastry factory workers, cereal handlers, confectioners, and bakery workers. It is considered one of the most frequent occupational, cereal-induced allergic asthmas. Consuming cooked wheat or products containing it does not manifest symptoms in these patients, but they may react after eating products contaminated with raw wheat flour.

IgE-mediated wheat allergens are widely distributed in wheat's different protein fractions. Currently, 28 allergens have been identified in wheat, according to WHO/IUIS Allergen Nomenclature Sub-Committee. The heat-resistant α-amylase/trypsin inhibitor is an allergen that binds to specific IgE and is involved in anaphylaxis, in some cases of WDEIA, and baker's asthma. Wheat seeds highly express Tri a 37, which is a plant defence protein. It is also resistant to digestion and heat and can act as a powerful allergen. Individuals who have IgE antibodies against Tri a 37 have a high risk of severe allergic symptoms upon wheat intake. ω-5-gliadin, also known as Tri a 19, is involved in anaphylactic reactions to wheat and WDEIA in children.

Non-IgE-Mediated wheat allergy

Non-IgE-mediated wheat allergy usually occurs 2 Hours after ingestion of wheat. It is strongly associated with eosinophilic esophagitis (EoE) or eosinophilic gastritis (EG), which occur when eosinophils infiltrate the gastrointestinal trac. Typical manifestations of this type of WA are indigestion, diarrhea, vomiting, arthralgia, and headaches that can appear numerous hours or days after consumption of allergens.

Wheat Allergen	Biochemical Name	Molecular Weight (kDa)	Route of Allergen Exposure
Tri a 12	Profilin	14	Food
Tri a 14	Non-specific lipid transfer protein 1	9	Food
Tri a 15	Monomeric alpha- amylase inhibitor 0.28	-	Airway
Tri a 17	Beta-amylase 56	56	Food
Tri a 18	Agglutinin isolectin 1	-	Food
Tri a 19	Omega-5 gliadin, seed storage protein	65	Food
Tri a 20	Gamma gliadin	35 to 38	Food

Unrecognized health benefits of gluten

Although limited in numbers, several studies suggest gluten itself may have health benefi ts in addition to its energy value as a protein. For example, serum triglycerides were reduced 13% in 24 adults with hyperlipidemia who consumed an additional 60 g/day of gluten over a 2-week period.48 In the same subjects, a diet with increased amounts of wheat fi ber or wheat bran had no effect on triglycerides. In another study by the same researchers, men and women with hyperlipidemia consumed either a control diet or a diet in which 11% of the carbohydrates were replaced with wheat gluten.

Over the 4-week study period, the increased gluten intake (78 g/day) reduced serum triglycerides by 19.2%, uric acid by 12.7%, and low-density lipoprotein (LDL) oxidation by 10.6%. The researchers noted that the observed effects of added wheat gluten on serum triglycerides and oxidized LDL are comparable to those of monounsaturated fat and soy protein. Gluten also may have a positive effect on BP. Various peptide fragments of gliadin extracted from wheat gluten have been shown to inhibit angiotensin-converting enzyme, which could help lower BP. Whole-grain diets have been shown to reduce BP in adults with mild hypercholesterolemia, with wheat having a specific effect. In addition to the contribution of the dietary fiber component of whole grains in BP regulation, gluten (via its constituent protein gliadin) also may play a role.

Gluten-free options

With the explosion of interest in gluten-free foods, more options are available, including gluten-free grains, seeds, flours, and starches that can help patients obtain adequate dietary fiber normally found in whole grains. These safe options are listed in Table below and may enable patients on a gluten-free diet to avoid some of the nutritional deficiencies, such as low intakes of thiamine, riboflavin, niacin, folate, and iron, that are associated with gluten avoidance. Gluten-free products also may have lower protein content than the gluten-containing foods they are designed to replace. By choosing foods that are nutrient-dense, patients on gluten-free diets can have well-balanced diets. This does not, however, mean that a gluten-free diet is a more healthful diet, as is commonly believed.

Patients who require a gluten-free diet should consult with a registered dietitian with expertise in celiac disease. Guidelines established by the FDA should help patients who require a gluten-free diet be able to determine whether a product really is gluten-free. To be labeled gluten-free, without gluten, free of gluten, or no gluten, the product must have fewer

than 20 parts per million of gluten. This level corresponds to the lower limit that can be consistently detected with available analytical tools, and is in agreement with recommendations in the scientific literature. Along with the growing numbers of people avoiding gluten, Internet sites catering to the demand for glutenfree products also have increased. More than 93% of patients with digestive diseases seek Web-based health information.

However, nearly one-half of 98 Internet sites that provided information on celiac disease had information that was judged to be less than 95% accurate. Furthermore, 20% of commercial, nonprofit, and professional websites provided information that was less than 90% accurate.

Gluten-free grains, seeds, flours, and starches

- Amaranth
- Arrowroot
- Buckwheat
- Flax
- Legume flours
- Mesquite flour
- Millet
- Montina (Indian rice grass)
- Nut flours
- Oats*
- Potato starch and flour
- Quinoa
- Rice (white, wild, brown, black, glutinous/sweet)
- Rice bran
- Sorghum
- Soy
- Sweet potato flour
- Tapioca
- Teff

AIM AND OBJECTIVE

Aim

Formulation, comparison and evaluation of gluten and gluten free cookies.

Objective

1. To Conduct Pre formulation Studies of the Cookies.
2. To Formulate Cookies With Gluten And Without Gluten.

3. Stability study of cookies.
4. Evaluation of gluten and gluten free value of cookies.

Formulation and Preparation

Ingredients used for preparation

1. Wheat
2. Oats
3. Flaxseeds
4. Almonds
5. Peanuts
6. Black pepper
7. Cinnamon
8. Jaggery
9. Walnuts
10. Butter

1. Wheat

Biological name- Triticum aestivum

Family- Poacea

Nutrition constituents: Starch, protiens, lipid, fiber, minerals, terpinoids, phenolic. Wheat is a grass widely cultivated for its seed, a cereal grain that is a worldwide staple food. Hence wheat is cultivated and harvested throughout the year in one country or other, China. India, Russianfederation, USA, France, Canada, Germany, Pakistan, Australia and Turkey are most important wheat growing countries.

Uses

1. Wheat has a several medicinal virtues -starch and gluten in wheat provide heat and

energy.

2. The inner barn coats, phosphate and other mineral salts.
3. It is rich in fiber which helps in the bowel movement.

2. Oats

Biological name- Avena Sativa

Family-Poaceae

Nutrition constituents: Proteins, Fat, Fiber, Carbohydrates, Calories, Lipids, Starch, Glucan, potassium. The grain refers specifically to the edible seeds of oat grass. Oats are mainly grown in Russia, Canada, Poland, Australia, and Finland etc.are the most oats growing countries.

Uses

1. Oats lowers blood sugar.
2. Provides antioxidants, promotes healthy bacteria in gut.
3. Eases constipation, helps in weight balance.
4. Relieves skin itching and irritation.

3. Flaxseeds

Biological name-Lignum usitatissium Linn

Family-Linaceae

Nutrition constituents: Proteins, Dietaryfibre, saturated fats, sodium, potassium, sugar, vitamin c, iron, vitamin B6,magnesium. flaxseed comes from the flax plant, which grows to be 2 feet tall. It likely was first grown in Egypt but has been cultivated all around the world. And started growing in other countries like Kazakhstan, Russia, Canada and China.

Uses

1. Flax seeds are also known as nutritional powerhouse.
2. It provide a good amount of fiber, protein, magnesium and manganese in each serving
3. The insoluble fibres in flaxseed helps to improve laxation and prevent constipation.
4. Flax seeds have abundance of ALA omegs-3 fatty acids.
5. Flax seeds have high content of lignans which acts as anti-oxidants.
6. One teaspoon of flax meal with 2.5 teaspoon of water to use handy substitute for one egg in baked goods.
7. With mild yet slightly nutty flavour, flaxseed also makes the perfect addition to cereals, oatmeal, Soups and salads.
8. It is used ad dietary fibre.
9. Used to cure diabetics.

4. Almonds

Biological name- Prunus dulcis

Family-Rosaceae

Nutrition constituents: Oleic acid, Fibre, Carbohydrates, Proteins, Lipids, healthy fats, Magnesium, Vitamin E. Almonds are edible nuts which mainly grown in the Spain, Italy, Australia, about of 80% come from California.

Uses

1. IT lowers Blood Sugar Level.
2. Reduces blood pressure and lower cholestrol level. Nourishes nerves system.
3. Almonds promote the weight loss. Increases memory power.
4. Almonds make teeth and bone strong, prevents OSTEOPROSIS.

5. Peanuts

Biological name-Arachis hypogaea

Family-Fabaceae

Nutrition constituents: Calcium, Fibre, Carbohydrates, Proteins, Lipids, healthy fats, Magnesium, Vitamin D, Vitamin C, Vitamin B6, Minerals, Folate. Peanuts are grown for edible seeds. Peanut plant are mainly grown in Asia, Africa, Australia, North and SouthA merica. More than half of the peanuts are grown in India and China.

Uses

1. Peanuts help to prevent heart disease by lowering cholestrol level.
2. They can also reduce the small blood clothes.
3. Peanuts also help in the brain improvement.
4. Helps in weight loss.
5. Reduces risk of cancer.

6. Cinnamon

Biological name-Cinnamomum zeylanicum

Family-Lauraceae

Nutrition constituents: Carbohydrates, Calcium, Iron, Antioxidants such as Polyphenols, Vitamin A. It is dried inner bark which is mainly grown in Srilanka, Malabar coast of India and also cultivated in South America and West indies.

Uses

1. It is the power house of Antioxidants.
2. It is the best remedies for tooth pain.
3. Treats the viral infections.
4. Helps in weight management.
5. Prevent type 2 diabetics.
6. Helps in infertility treatment.

7. Black pepper

Biological name- Pipper nigrum

Family-Piperaceae

Nutrition constituents: Carbohydrate, fat, Minerals and high level of Antioxidants. It is cultivated as a fruit, Dried, used as a spice and seasoning. They are grown in Brazil, Indonesia, India, Srilanka.

Uses

1. Black pepper has anti inflammatory properties.
2. Improve blood sugar levels.
3. May lower cholestrol level.
4. Helps in weight loss.
5. Fights with bacterial infections

8. Jaggery

Jaggery is made from the juices of palm trees or sugar cane and is growing in popularity as a replacement for white sugar.

Nutrition constituents

Iron, Vitamin B12, Vitamin B6, Calcium, Folate, Selenium, Magnesium.

Uses

1. Prevention of respiratory problems.
2. Helps with weight loss.
3. Mainly great source of energy as it contains Iron
4. It prevents Ananemia, It purifies the body.
5. And also Detoxification of the Liver.

9. Walnuts

Biological name- Juglans regia.

Family – Juglandaceae.

Nutrition constituents: Proteins, Fiber, Fat, Calories, Carbohydrates, Sugar, Biotin. They grown in north and south America, southern Europe, asia and the west indies.

Uses

1. Rich in antioxidant.
2. Reduce the risk of some cancers.
3. Support weight control.
4. Lowers Blood Pressure.

9. Butter

Butter is a yellow to white solid emulsion of fat globules, water and inorganic salts produced by churning the cream from milk.

Nutrition constituents: Calories, Fats, Sodium, Vitamin A, Vitamin E, Calcium.

Uses

1. It can help lower the chances of Cancer.
2. Butter is high in Beta-Carotene, a compound that converts into Vitamin A
3. It helps to make skin healthier.
4. It is also used as a lubricant.

Formulation

Quantity of ingridents

Ingredients	Formulation 1 (F1)	Formulation 2 (F2)	Formulation 3 (F3)
Oats flour	40g	-	20g
Wheat flour	-	40g	20g
Flaxseeds flour	20g	20g	20g
Almond flour	20g	20g	20g
Peanut flour	20g	20g	20g
Walnut flour	10g	10g	10g
Cinnamon powder	0.2g	0.2g	0.2g
Black peeper powder	01g	01g	01g
Jaggery syrup	30ml	30ml	30ml
Butter	1 Tea spoon	1 Tea spoon	1 Tea spoon

Procedure for nutritive cookies

1) Preparation of F1 formulation

Weight approximately 40g of Oats flour.

Add 20g of flaxseeds flour, peanut flour, and almond flour respectively.

And then add 10g of walnut flour.

Weigh 0.2g of cinnamon powder and 1g of black pepper powder.

Mix all the flours uniformly.

Slowly add the 30 ml Jaggery syrup by continuous stirring.

Make a dough formation by adding 1 table spoon of butter as a lubricant.

After dough formation cut the dough into small pieces of 20g each.

Make the dough pieces into a uniform shape.

Keeps the oven for pre heating.

Place them evenly apart on a baking sheet.

Maintain the oven temperature to 170 degree for 20 min.

Remove the baked cookies from the oven and allow it to stand for room temperature.

2) Preparation of F2 formulation

Weight approximately 40g of Wheat flour.

Add 20g of flaxseeds flour, peanut flour, almond flour respectively.

And then add 10g of walnut flour.

Weigh 0.2g of cinnamon powder and 1g of black pepper powder.

Mix all the flours uniformly.

Slowly add the 35 ml Jaggery syrup by continuous stirring.

Make a dough formation by adding 1 table spoon of butter as a lubricant.

After dough formation cut the dough into small pieces of 20g each.

Make the dough pieces into a uniform shape.

Keep the oven for pre heating.

Place them evenly apart on a baking sheet.

Maintain the oven temperature to 170 degree for 20 min.

Remove the baked cookies from the oven and allow it to stand for room temperature.

3) Preparation of F3 formulation

Weight approximately 20g of Oats flour and Wheat flour.

Add 20g of flaxseeds flour, peanut flour, almond flour respectively.

And then add 10g of walnut flour.

Weigh 0.2g of cinnamon powder and 1g of black pepper powder.

Mix all the flours uniformly.

Slowly add the 30 ml Jaggery syrup by continuous stirring.

Make a dough formation by adding 1 table spoon of butter as a lubricant.

After dough formation cut the dough into small pieces of 20g each.

Make the dough pieces into a uniform shape.

Keep the oven for pre heating.

Place them evenly apart on a baking sheet.

Maintain the oven temperature to 170 degree for 20 min.

Remove the baked cookies from the oven and allow it to stand for room temperature.

Testings

Testings for cookies

1. Preliminary test

A) Colour –Goldenish Brown

B) Taste-Sweet and Spicy

C) Odour-Aromatic

Preformulation studies

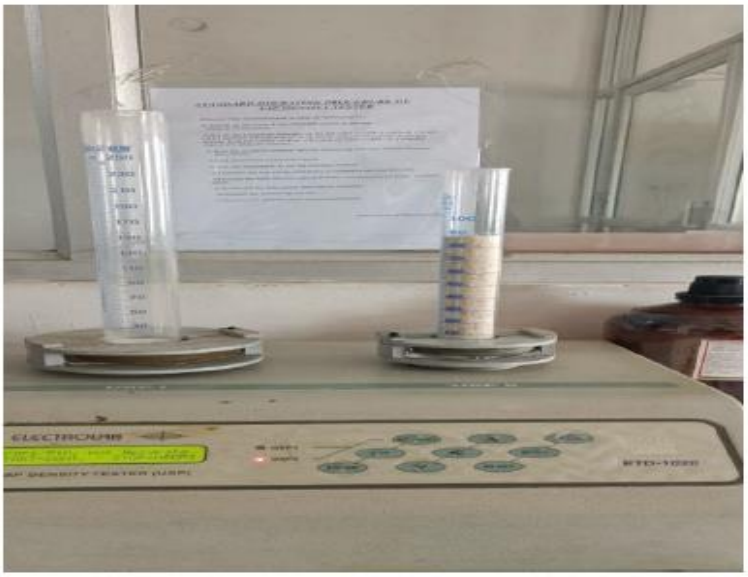
1. Tap Density
2. Bulk Density
3. Angle of Repose

1. Tap density

The tap density is a increased bulk density attend after mechanically tapping a container containing the powder sample.

$$\text{Tap density} = \frac{\text{Weight of sample}}{\text{Tapped Volume}}$$

$$\text{OR Tap density} = \frac{100(\text{Volume(in)} - \text{Volume(F)})}{\text{Volume(in)}}$$



Apparatus used to test tap den

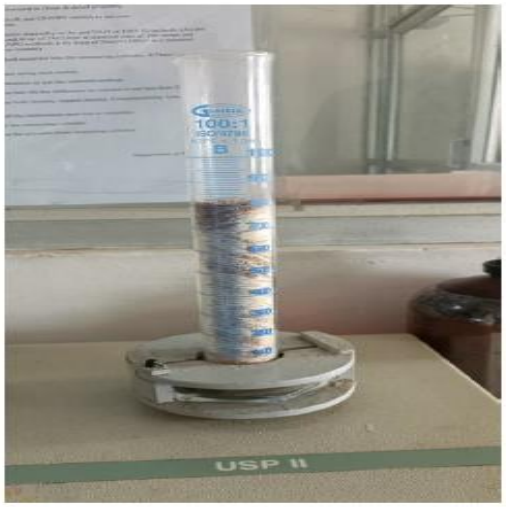
Sl no	Sample	No of Taps	Tap density		Tapped Volume (In-Fin)
			Inital	Final	
01	Formulation F1	50	81	64	17
02	Formulation F2	50	88	77	11
03	Formulation F3	50	82	68	14

(A) Formulation 1 (F1)

The initial volume of material = 81 The final volume of material = 64 Tap density = ??

$$\text{Tap density} = \frac{\text{Weight of sample}}{\text{Tapped Volume}}$$

$$= \frac{35}{17} = 2.05 \text{ g/ml}$$



Initial volume

Final volume

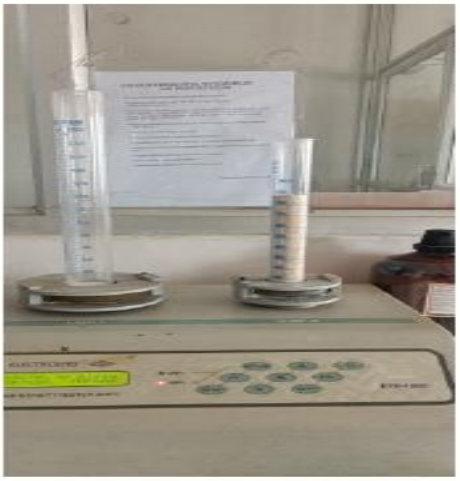
(B) Formulation 2 (F2)

The initial volume of material = 88 The final volume of material = 77 Tap density = ??

$$\text{Tap density} = \frac{\text{Weight of sample}}{\text{Tapped Volume}}$$

$$= \frac{35}{11} = 3.181 \text{g/ml}$$

Initial volume



Final volume

(C) Formulation 3 (F3)

The initial volume of material = 82 The final volume of material = 68 Tap density = ??

$$\text{Tap density} = \frac{\text{Weight of sample}}{\text{Tapped Volume}}$$

$$= \frac{35}{14} = 2.5 \text{ g/ml}$$

Initial volume

Final volume

2. Bulk density

The bulk density value includes rhe volume of all of the pores within the powedr of sample.

$$\text{Bulk density} = \frac{\text{Weight of powder}}{\text{Volume of powder}}$$

(A) Formulation 1 (F1): Weight of material = 35g Volume of material = 81 Bulk density = ??

$$\text{Bulk density} = \frac{\text{Weight of powder}}{\text{Volume of powder}}$$

$$= \frac{35}{81} = 0.432 \text{g/ml}$$

(B) Formulation 2 (F2)

Weight of material = 35g Volume of material = 88 Bulk density = ??

$$\text{Bulk density} = \frac{\text{Weight of powder}}{\text{Volume of powder}}$$

$$= \frac{35}{88} = 0.397 g/ml$$

(C) Formulation 3(F3)

Weight of material = 35g Volume of material = 82 Bulk density = ??

$$\text{Bulk density} = \frac{\text{Weight of powder}}{\text{Volume of powder}}$$

$$= \frac{35}{82}$$

$$= 0.426 g/ml$$

Final bulk density

Sl. no.	Formolation	Bulk density
01	F1	0.432g/ml
02	F2	0.397g/ml
03	F3	0.426g/ml

Angle of repose

The angle of repose is a relatively simple technique for estimating the flowability of a powder. The angle of repose can be determined by experimentally by allowing a powder to flow through a funnel and fall freely onto a surface.

The height and diameter of the resulting cone is measured.

It is the maximum angle that can be obtained between the free standing surface of a powder heap and the horizontal plane.

1. F1 Formulation

The diameter obtained from the angle of repose is D1= 6.4cm = R1 = 3.2cm (D = R/2) D2= 6.9cm = R2 = 3.4cm D3= 6.1cm = R3 = 3.0cm

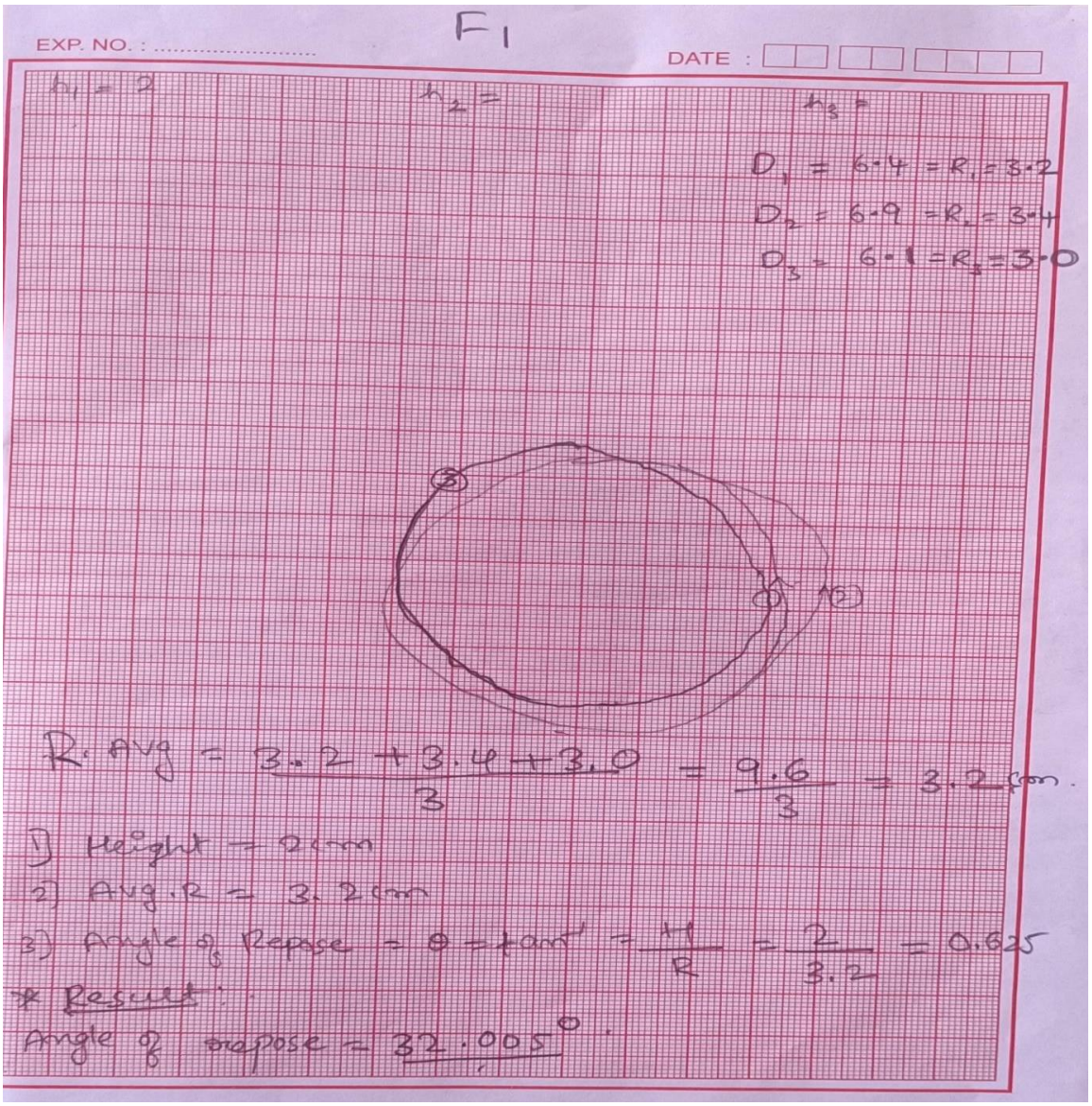
The obtained average radius is

$$= \frac{3.2+3.4+3.0}{3} = \frac{9.6}{3} = 3.2cm$$

1) Height = 2cm
2) Average radius = 3.2cm
3) Angle of repose = $\tan\theta = \frac{H}{R} = \frac{2}{3.2} = 0.625$

Results

Angle of repose = 32.0005˚.



2. F2 Formulation

The diameter obtained from the angle of repose is D1= 6.3cm = R1 = 3.1cm (D = R/2) D2= 6.4cm = R2 = 3.2cm D3= 6.9cm = R3 = 3.4cm.

The obtained average radius is

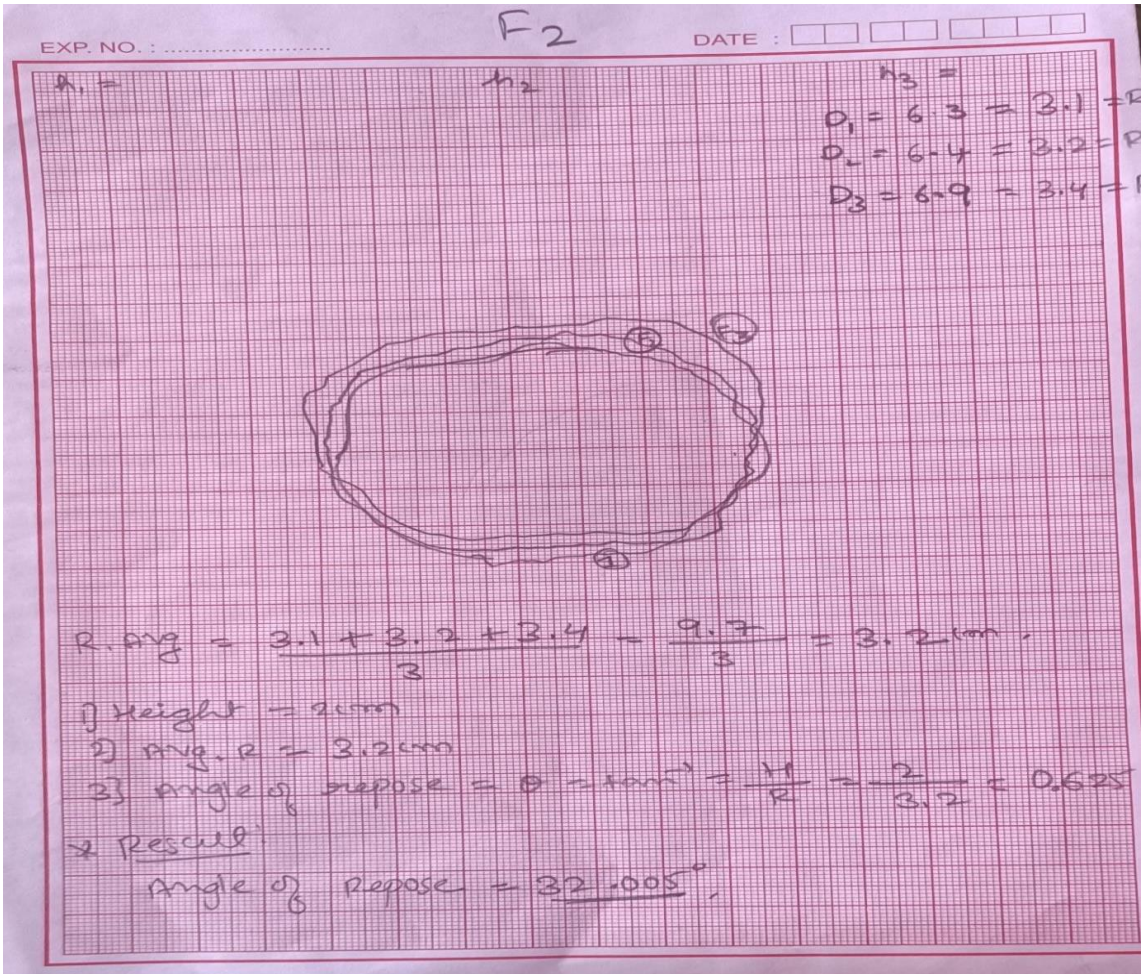
$$= \frac{3.1+3.2+3.4}{3} = \frac{9.7}{3} = 3.2\text{cm}$$

1) Height = 2cm
2) Average radius = 3.2cm

3) Angle of repose $= \tan\theta = \frac{H}{R} = \frac{2}{3.2} = 0.625$

Results

Angle of repose = 32.0005˚.



3. F3 Formulation

The diameter obtained from the angle of repose is D1= 6.0cm = R1 = 3.0cm (D = R/2) D2= 6.2cm = R2 = 3.1cm D3= 6.0cm = R3 = 3.0cm

The obtained average radius is

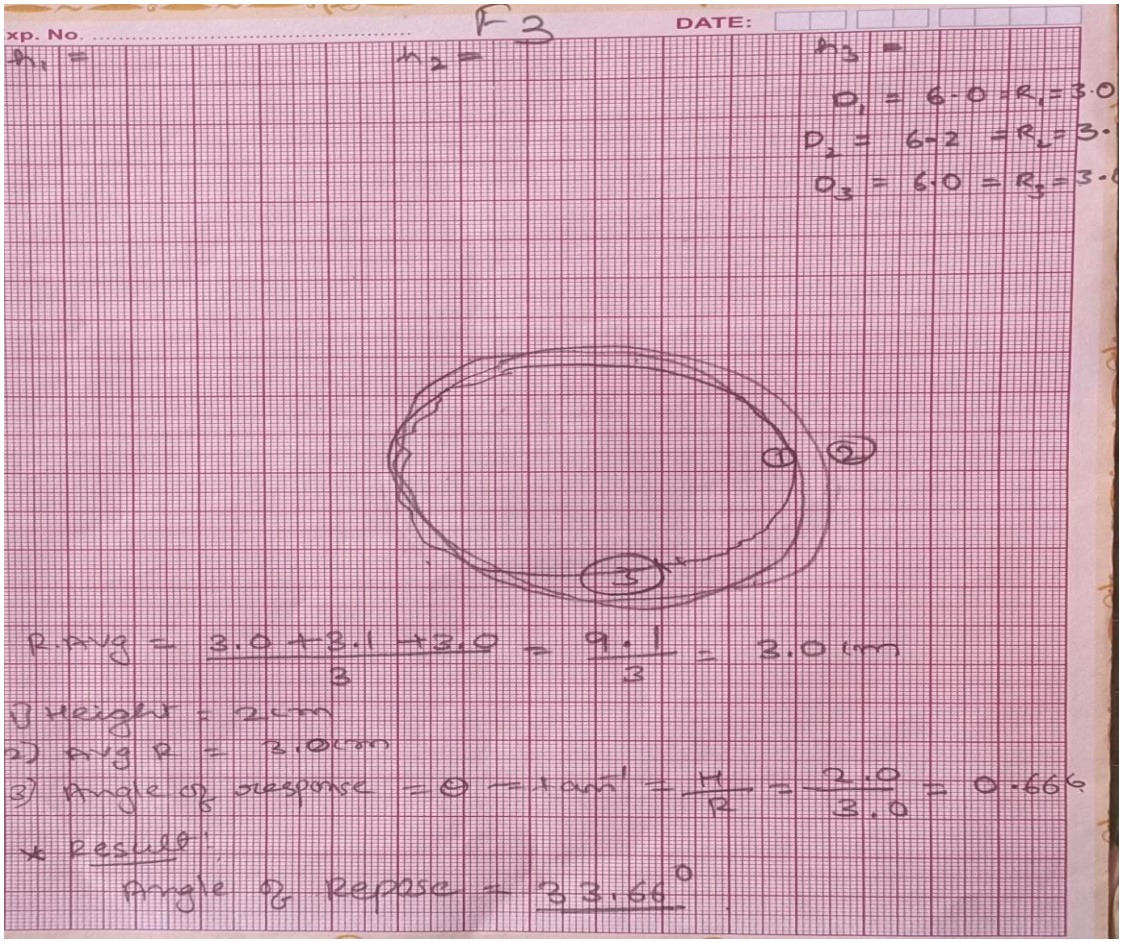
$= \frac{3.0+3.1+3.0}{3} = \frac{9.1}{3} = 3.0\text{cm}$

1) Height = 2cm
2) Average radius = 3.0cm

3) $\text{Angle of repose} = \tan\theta = \frac{H}{R} = \frac{2}{3.0} = 0.666$

Results

Angle of repose = 33.66˚.



Qualitative tests

1. F1 Formulation

Test for the carbohydrates

Test	Observation	Interference
A) Molish Test Take 2 ml of sample. Add 4 drops of Molish reagent. And add 1ml of H2SO4 Along the side of the test.	A purple ring is formed at The junction of two layers.	May be carbohydrate.
B) Bendict's test To 5ml of Benedict's reagent Add 4-5 drops of sample solution and heat it.	No Red PPT is formed.	Reducing sugar present may be a starch.

Test for proteins

Test	Observation	Interference
A) Ninhydrin test Take a 2 ml of sample solution. Add 1-2 ml of Nitric acid.	Yellow colour turns to Orange.	Proteins may be present.
B) Biuret test Add 2ml of NaOH and 5 to 6drops of copper sulphate solution to it. Shake the test tube and allow it to stand for 4-5 minutes	Appearance of Bluish to Violet colour.	Confirms the presence of proteins.

Test for fats

Test	Observation	Interference
A) Filter paper test In a filter paper, take a small quantity of the food sample. Leave the experiment undisturbed for 3 to 5 min. Keep near the sunlight or near the window.	Oily patches are formed on the filter paper	Presence of fats.
B) Emulsion test Take a food sample. Add 2-3 ml of ethanol and shake it. Allow the test tube to stand for 2 minutes. Take 2-3 ml of distilled water in a test tube and add the above solution	Milky – White Emulsion is formed.	Confirms the presence of Fats.

2. F2 formulation

Test for the carbohydrates

Test	Observation	Interference
A) Molish test Take 2 ml of sample. Add 4 drops of Molish reagent. And add 1ml of H2SO4 Along the side of the test.	A purple ring is formed at The junction of two layer.	May be carbohydrate.
B) Bendict's test To 5ml of Benedict's reagent Add 4-5 drops of sample solution and heat it.	No Red PPT is formed.	Reducing sugar present may be a starch.

Test for proteins

Test	Observation	Interference
A) Ninhydrin test Take a 2 ml of sample solution.	Yellow colour turns to Orange.	Proteins may be presnt.

Add 1-2 ml of Nitric acid.		
B) Biuret test Add 2ml of NaOH and 5 to 6drops of copper sulphate solution to it. Shake the test tube and allow it to stand for 4-5 minutes	Appearance of Bluish to Violet colour.	Confirms the presence of proteins.

Test for fats

Test	Observation	Interference
A) Filter paper test In a filter paper, take a small quantity of the food sample. Leave the experiment undisturbed for 3 to 5 min. Keep near the sunlight or near the window.	Oily patches are formed on the filter paper	Presence of fats.
B) Emulsion test Take a food sample. Add 2-3 ml of ethanol and shake it. Allow the test tube to stand for 2 minutes. Take 2-3 ml of distilled water in a test tube and add the above solution	Milky – White Emulsion is formed.	Confirms the presence of Fats.

3. F3 Formulation

Test for the carbohydrates

A) Molish test Take 2 ml of sample. Add 4 drops of Molish reagent. And add 1ml of H2SO4 Along the side of the test.	A purple ring is formed at The junction of two layer.	May be carbohydrate.
B) Bendict's test To 5ml of Benedict's reagent Add 4-5 drops of sample solution and heat it.	No Red PPT is formed.	Reducing sugar present may be a starch.

Test for proteins

Test	Observation	Interference
A) Ninhydrin test Take a 2 ml of sample solution. Add 1-2 ml of Nitric acid.	Yellow colour turns to Orange.	Proteins may be present.
B) Biuret test Add 2ml of NaOH and 5 to 6drops of copper sulphate solution to it.	Appearance of Bluish to Violet colour.	Confirms the presence of proteins.

Shake the test tube and allow it to stand for 4-5 minutes		

Test for fats

Test	Observation	Interference
A) Filter paper test In a filter paper, take a small quantity of the food sample. Leave the experiment undisturbed for 3 to 5 min. Keep near the sunlight or near the window.	Oily patches are formed on the filter paper	Presence of fats.
B) Emulsion test Take a food sample. Add 2-3 ml of ethanol and shake it. Allow the test tube to stand for 2 minutes. Take 2-3 ml of distilled water in a test tube and add the above solution	Milky – White Emulsion is formed.	Confirms the presence of Fats.

Quantitative test

1. Test for gluten in wheat flour

Procedure

Weigh 40g of wheat flour.

⬇

Prepare the wheat flour dough.

⬇

After the dough formation, keep the beaker filled with water and let the dough stand for 1 hour.

⬇

Transfer the dough in a sieve (number 125, MIC: 125).

⬇

Wash the dough with a tap water and block the sink.

⬇

Allow the water to stand in the sink.

⬇

Add few drops of iodine solution in the sink, if blue colour indicates wash the dough again.

⬇

Wash the dough upto no blue colour indication.

⬇

If no blue colour absorbed it indicates the presence of gluten.

Drying of glutein extraction

Note the empty petridish weight.

⬇

Cut the gluten into small pieces in petri dish.

⬇

Dry the gluten in hot air oven for 133degree for 2 hours.

⬇

After drying, cool the gluten in desiccator for 15 min.

⬇

Take out the dry gluten and note down the weight.

Dried glutein

Calculation

Formula

$$\text{Gluten percentage} = \frac{W2\text{-}W1}{Ws} \times 100$$

W1 =Petri dish weight

W2 =petri dish +gluten extraction weight Ws =Sample weight

Therefore: W1=37.948g W2=42.510g Ws=40.00g

Then,

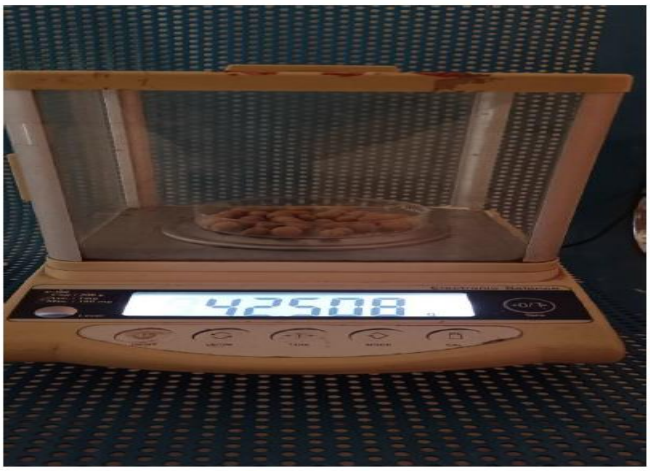
$$\text{Gluten percentage}=\frac{42.510\text{g}-37.948\text{g}}{40.00\text{g}}\times 100$$

$$=\frac{4.562}{40.00}\times 100$$

Gluten percentage=11.405%.

So in 40g of wheat flour it contains 11.405% of gluten content.

Dried gluten extraction



Ash value test

Test for F1 Sample

Note the empty crucible weight

⬇

Weight 2g of Sample F1

⬇

Add 2-4 drops of Conc H2SO4 (Sulfuric Acid) with fumes

⬇

Place the sample in hot air oven

⬇

Burn it for 1 hour at 550-600˚c

⬇

Remove the crucible and allow it stand for room temperature

⬇

Weight and note the crucible weight

⬇

Repeat the process up to the formation of complete ASH

Test for F2 Sample

Note the empty crucible weight

⬇

Weight 2g of Sample F2

⬇

Add 2-4 drops of Conc H2SO4 (Sulfuric Acid) with fumes

⬇

Place the sample in hot air oven

⬇

Burn it for 1 hour at 550-600˚c

⬇

Remove the crucible and allow it stand for room temperature

⬇

Weight and note the crucible weight

⬇

Repeat the process up to the formation of complete ASH

Test for F3 Sample

Note the empty crucible weight

⬇

Weight 2g of Sample F3

⬇

Add 2-4 drops of Conc H2SO4 (Sulfuric Acid) with fumes

⬇

Place the sample in hot air oven

⬇

Burn it for 1 hour at 550-600˚c

⬇

Remove the crucible and allow it stand for room temperature

⬇

Weight and note the crucible weight

⬇

Repeat the process up to the formation of complete ASH

Total ash value

Sl no	Formulation	Sample+ Crucible weight 1st ovening	Sample+ Crucible weight 2nd ovening
01	F1	26.864g	26.711g
02	F2	. 26.648g	26.508g
03	F3	26.956g	26.842g

Moisture content test

Sample F1

Weigh F1 Cookie

⬇

Heat in an oven at 175 C For 30 minutes

⬇

Cool at room temperature in a desiccator

⬇

Weigh dry cookie

Sample F2

Weigh F2 Cookie

⬇

Heat in an oven at 175C For 30 minutes

⬇

Cool at room temperature in a desiccator

⬇

Weigh dry cookie

Sample F3

Weigh F3 Cookie

⬇

Heat in an oven at 175 C For 30 minutes

⬇

Cool at room temperature in a desiccator

⬇

Weigh dry cookie

Stability testing

The cookies were kept in air tight container for 2 months at normal room temperature.

Sl no	Formolation	Stability
01	F1	Stable at the room temperature for more than 2 months.
02	F2	Stable at the room temperature for more than 2 months.
03	F3	Stable at the room temperature for more than 2 months.

RESULTS AND DISCUSSION

A) Preformulation studies

1) Tap density test

Sl no	Formolation	Tap density
01	F1	2.05 g/ml
02	F2	3.18 g/ml
03	F3	2.50 g/ml

2) Final bulk density

Sl no	Formolation	Bulk density
01	F1	0.432g/ml
02	F2	0.397g/ml
03	F3	0.426g/ml

3) Angle of repose

Sl no	Formolation	Angle of repose
01	F1	32.005˚
02	F2	32.005˚
03	F3	33.666 ˚

B) Qualitative studies

1) Test for carbohydrates

The protein is performed to check the availability of protein by two tests.

a) Molish test

Sl no	Formolation	Observation	Interference
01	F1	A purple ring is formed at the junction.	May be carbohydrate are present.
02	F2	A purple ring is formed at the junction.	May be carbohydrate are present.
03	F3	A purple ring is formed at the junction.	May be carbohydrate are present.

b) Bendict's test

Sl no	Formolation	Observation	Interference
01	F1	No red ppt is formed.	Reducing sugar present may be starch.
02	F2	No red ppt is formed.	Reducing sugar present may be starch.
03	F3	No red ppt is formed.	Reducing sugar present may be starch.

2) Test for proteins

a) Ninhydrin test

Sl no	Formolation	Observation	Interference
01	F1	Yellow colour turns to orange.	Proteins may be present.
02	F2	Yellow colour turns to orange.	Proteins may be present.
03	F3	Yellow colour turns to orange.	Proteins may be present.

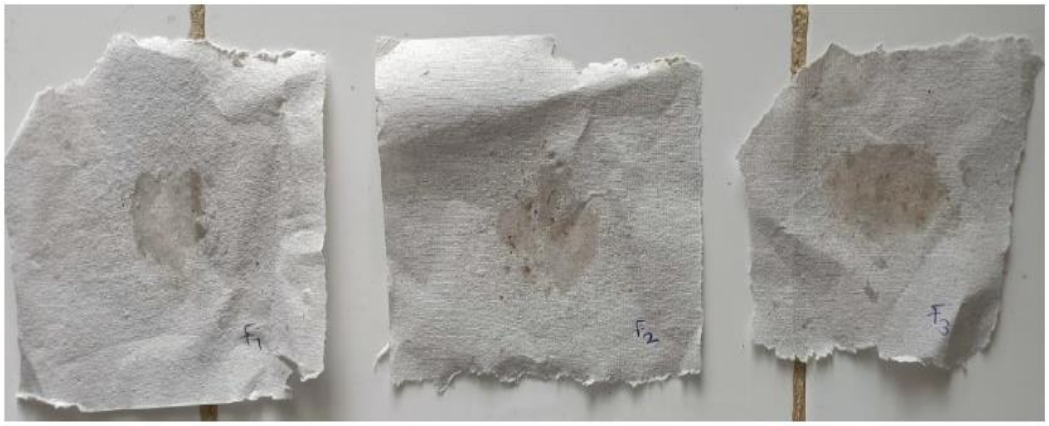
Biuret test

Sl no	Formolation	Observation	Interference
01	F1	Appearance of bluish to violet colour.	Confirms the presence of proteins.
02	F2	Appearance of bluish to violet colour.	Confirms the presence of proteins.
03	F3	Appearance of bluish to violet colour.	Confirms the presence of proteins.

3) Test for fats

a) Filter paper test

Sl no	Formolation	Observation	Interference
01	F1	Oily patches are formed on the filter paper.	Presence of fats.
02	F2	Oily patches are formed on the filter paper.	Presence of fats.
03	F3	Oily patches are formed on the filter paper.	Presence of fats.



b) Emulsion test

Sl no	Formolation	Observation	Interference
01	F1	Milky-White emulsion is formed.	Confirms the presence of fats.
02	F2	Milky-White emulsion is formed.	Confirms the presence of fats
03	F3	Milky-White emulsion is formed.	Confirms the presence of fats

Quantitative studies

a) Percentage of gluten in wheat flour

$$\text{Gluten percentage} = \frac{W2\text{-}W1}{Ws} \times 100$$

W1 =Petri dish weight

W2 =petri dish +gluten extraction weight Ws =Sample weight

Therefore:

W1=37.948g W2=42.510g Ws=40.00g

Then,

$$\text{Gluten percentage} = \frac{42.510g\text{-}37.948g}{40.00g} \times 100$$

$$= \frac{4.562}{40.00} \times 100$$

Gluten percentage=11.405%.

So in 40g 0f wheat flour it contains 11.405% of gluten content.

b) Ash value test

Empty crucible weight = 25.385. Sample = 2g.

Sl no	Formolation	Sample+ crucible weight 1st ovening	Sample+ crucible weight 2nd ovening
01	F1	26.864g	26.711g
02	F2	.26.648g	26.508g
03	F3	26.956g	26.842g

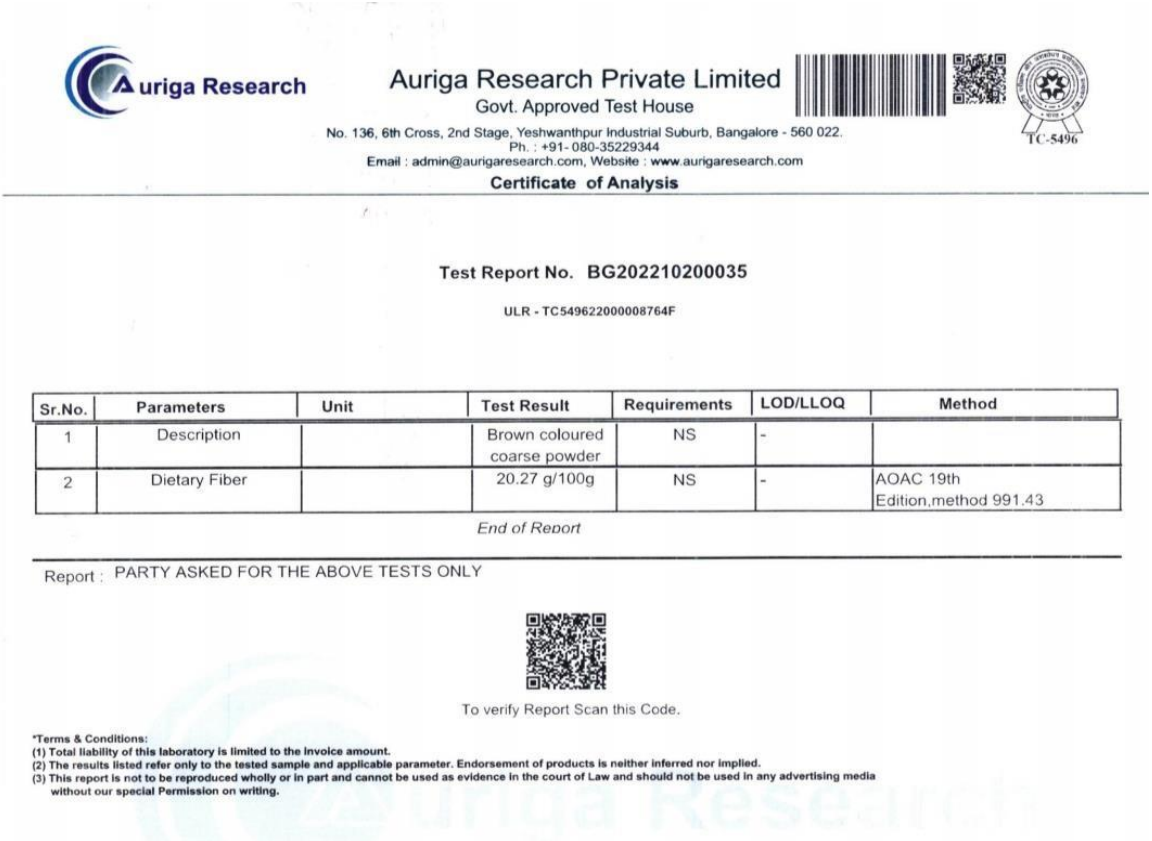
c) Moisture content test

Sl no	Formulation	Weight of cookies before drying	Weight of cookies after drying
01	F1	20g	18.699g
02	F2	.20g	18.568g
03	F3	20g	18.754g

d) Stability testing

Sl no	Formulation	Stability
01	F1	Stable at the room temperature for more than 2 months.
02	F2	Stable at the room temperature for more than 2 months.
03	F3	Stable at the room temperature for more than 2 months.

Percentage of dietry fiber



Auriga Research

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Email : admin@aurigaresearch.com, Website : www.aurigaresearch.com
TC-5496

Certificate of Analysis

Test Report No. BG202210200035

ULR - TC549622000008764F

Sr.No.	Parameters	Unit	Test Result	Requirements	LOD/LLOQ	Method
1	Description		Brown coloured coarse powder	NS	-	
2	Dietary Fiber		20.27 g/100g	NS	-	AOAC 19th Edition,method 991.43

End of Report

Report : PARTY ASKED FOR THE ABOVE TESTS ONLY

To verify Report Scan this Code.

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CONCLUSION

By the conclusion of the three formulations it concluded that the

F1 [oats]:

Oats having a high amount of beta glucan, and rich in fibre content so the F1 formulation oats is beneficial for the control of the diabetes and maintains the cholesterol levels.

It concluded that the use of the F1 formulation oats nutritive cookies is more beneficial for the

patients with diabetes, obesity and cardiovascular diseases.

F2:

Wheat is having a major source of fibre content and it having gluten in it, it is beneficial for the bowl moment and constipation.

The F2 formulation wheat is rich in energy production and gluten content in it helps for the dietary balance for the people.

F3:

The combination of the wheat and oats helps in the balancing energy levels, nutritive levels and digestion levels.

The F3 formulation oats and wheat is mainly beneficial for the geriatrics and pediatrics.

By comparison of the three formulations F1 (oats), F2 (wheat), and F3 (oats and wheat) combination it concluded that the high amount of gluten consumption is having adverse effects for the health.

As the above tests we performed, it concluded that the F2(wheat) and F3(wheat +oats) contains a gluten. we have found 11.45g of gluten in the F2 formulation, so as according to studies 7-9% of gluten is beneficial for health.

As the F1(oats) formulation is gluten free, it is rich in dietary fiber, low in calories and having good physical and sensory characteristics and thus can be developed as functional food.

By these comparison it concluded that the F1 formulation(oats) is having a high health benefits.

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