

CHARACTERIZATION OF β -GLUCAN FROM OYSTER MUSHROOM (*PLEUROTUS
PULMONARIUS*) AND ITS APPLICATION IN MODEL FOOD



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(*PLEUROTUS PULMONARIUS*) AND ITS APPLICATION IN MODEL FOOD

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ABSTRACT

CHARACTERIZATION OF β -GLUCAN OF OYSTER MUSHROOM (*PLEUROTUS PULMONARIUS*) AND ITS APPLICATION MODEL FOOD

Mushrooms are known as β -glucan sources. The purpose of this investigation was to characterize and purify β -glucan by alkaline extraction from oyster mushrooms (*Pleurotus pulmonarius*). in order to evaluate its molecular weight and viscosity. The ideal extraction process parameters were found as 80°C, 90 minutes, and 30% KOH. FT-IR analysis, ^1H and ^{13}C NMR spectroscopy, were used for chemical characterization and extracted β -glucan was found to be a β -(1-3) glucose polymer with β -(1-6) side chains. The molecular weight (Mw) was determined as 349.260 g/mol. Rheological measurements showed that β -glucan has low apparent viscosity in aqueous solutions. No microbial action was determined by testing the antibacterial activity of β -glucan against *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, and *Enterococcus hirae* ATCC 10541 using the disc diffusion technique. Lastly, β -glucan was added to the cracker recipe, and the finished product's sensory and physical characteristics as well as its estimated glycemic index were assessed. The glycemic index of β -glucan crackers ranged from 88.07 to 67.32, whereas the glycemic index of glucose was 100. The findings of the sensory study led to the conclusion that crackers supplemented with β -glucan and having functional qualities may satisfy consumer expectations.

ÖZET

B-GLUKANIN İSTİRİDYE MANTARINDAN (*PLEUROTUS PULMONARIUS*) KARAKTERİZASYONU VE MODEL GIDADA UYGULANMASI

Mantarlar β -glukan kaynakları olarak bilinir. Bu çalışmanın amacı, molekül ağırlığını ve viskozitesini değerlendirmek için istiridye mantarlarından (*Pleurotus pulmonarius*) alkali ekstraksiyon yoluyla β -glukanı karakterize etmek ve saflaştırmaktır. İdeal ekstraksiyon proses parametreleri 80°C, 90 dakika ve %30 KOH olarak bulunmuştur. Kimyasal karakterizasyon için FT-IR analizi, ^1H ve ^{13}C NMR spektroskopisi kullanıldı ve ekstrakte edilen β -glukanın β -(1—6) yan zincirleri olan bir β (1—3) glikoz polimeri olduğu bulundu. Molekül ağırlığı (Mw) 349.260 g/mol olarak belirlendi. Reolojik ölçümler, β -glukanın sulu çözeltilerde düşük görünür viskoziteye sahip olduğunu göstermiştir. Disk difüzyon tekniği kullanılarak *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739 ve *Enterococcus hirae* ATCC 10541'e karşı β -glukanın antibakteriyel aktivitesi test edilerek hiçbir mikrobiyal etki belirlenmemiştir. Son olarak, kraker reçetesine β -glukan eklenmiş ve bitmiş ürünün duyuusal ve fiziksel özellikleri ile tahmini glisemik indeksi değerlendirilmiştir. B-glukanlı krakerlerin glisemik indeksi 88.07 ile 67.32 arasında değişirken, glukozun glisemik indeksi 100'dür. Duyusal çalışmanın bulguları, β -glukan takviyeli ve fonksiyonel niteliklere sahip krakerlerin tüketici beklentilerini karşılayabileceği sonucuna varılmıştır.

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LIST OF SYMBOLS/ABBREVIATIONS

AACC	American Association of Cereal Chemists
ABTS	(2,2' azinobis-(3-Ethylbenthiazolin -6-sulfonic acid)
ALT	Alanine transaminase
AMG	Amiloglikozidase enzyme
AST	Aspartate transaminase
BMIMCl	1-Butyl-3methylimidazolium chloride
C ₅ H ₅ N	Pyridine
CCNFSDU	Codex committee on nutrition and foods for special dietary uses
CHD	Coronary heart disease
CP	Cross polarization
CP-MAS	Cross polarization magic angle spinning
CR3	Complement receptor 3
D ₂ O	Deuterium oxide
DMSO	Dimethyl sulfoxide
DPPH	2,2 diphenyl-1-picrylhydrazyl
DSHEA	Dietary supplement health and education act
eGI	Estimated glycemic index
EURODIAD	European insulin dependent diabetes prevention study
FOS	Fructooligosaccharide
GC_MS	Gas chromatography-mass spectrophotometer
GM	Galactomannan
HbA1C	Hemoglobin
HCl	Hydrochloric acid
HI	Hydrolysis index
HPLC	High pressure liquid chromatography
HSO ₃ Cl	Chlorosulfuric acid
IDF	Insoluble dietary fiber
KBr	Potassium bromide
KOH	Potassium hydroxide
LC-MS	Liquid-chromatography-mass spectrometry

MAS	Magic angle spinning
MW	Molecular weight
NMR	Nuclear magnetic resonance
POCl ₃	Phosphoryl chloride
RAG	Rapid available glucose
RRLC	Rapid resolution liquid chromatography
RS	Resistant starch
SAG	Slowly available glucose
SDF	Soluble dietary fiber
SEM	Scanning electron microscopy
SWM	Solid waste management
T2DM	Type 2 diabetes mellitus
TDF	Total dietary fiber
WHO	World health organization

1. INTRODUCTION

According to the definition of the World Health Organization (WHO), Diabetes Mellitus (DM) is a chronic metabolic disease characterized by irregular carbohydrate, lipid and protein metabolism, characterized by hyperglycemia, which may occur due to many causes and is caused by a disorder in insulin release and/or activity [1]. It has been reported by the WHO that DM, whose prevalence is increasing both in Turkey and in the world, is the biggest health disaster related to chronic disease ever seen in the world. DM is included in 'epidemic diseases' group [2]. Over the past century, the food industry has produced products that are attractive in taste and appearance, high in energy density but low in nutritional value. The incorrect, inadequate, and unbalanced eating habits developed in individuals with the combination of these products, which are produced by the food industry for profit, with obesogenic environmental conditions have resulted in an increase in health problems [3]. Today, societies and individuals have become more aware of healthy nutrition and have started to regard it more importantly for their daily nutrition. The use of natural resources to treat DM and the innovation of new drug molecules based on these resources started in the first quarter of the 20th century. In many regions in the world, various plants and nutrients are used traditionally to treat DM. Some of these traditional treatments are considered by scientific circles and WHO supports the studies in this field [4]. Especially in recent years, the increase in chronic diseases and the desire to have a quality life have led individuals to functional foods [3].

In today's world conditions, as living conditions have become easier and living standards have increased in many countries, people have become more sensitive about the properties and health effects of the food they buy. Today, consumers are looking at the additional benefits of the food they consume rather than just nutrition. As people have become more sensitive about food, manufacturers have increased their search for new products and their interest in functional foods. People want to find solutions to their ailments naturally at home and protect their health in these natural ways rather than spending their time in health institutions as they used to. The reason is that despite the side effects of drugs, the reality that functional foods do not lead any side effects if taken in appropriate doses and conditions is one of the main reasons that drive consumers to use functional foods [5]. Consumers are

attracted by the use of functional foods for both nutritional purposes and for treating disease and maintaining health. Therefore, interest in functional foods is increasing every passing day.

1.1. DEFINITION AND COMPONENTS OF FUNCTIONAL FOODS

When present definitions of functional food over the world are considered, there is no agreed definition. The legislation of the Ministry of Agriculture and Forestry in Turkey defines functional foods as follows: In addition to their nutritional effects, they are defined as foods that have health-protective, corrective and/or disease risk-reducing effects depending on one or more effective components and these effects have been scientifically and clinically proven [6]. According to the Japan Ministry of Health, Labor and Welfare, functional foods are: “FOSHU” (use of food for health) must contain a food component that the health of the body benefited, along with its claimed effects on human health must be scientifically validated. The foods in the “FOSHU” class are intended to be consumed by people who want to support and maintain health or who want to control diseases such as diabetes, cholesterol, rheumatism [7]. According to the European Commission, nutrients that provide additional benefits for the health of the body beyond their nutritional function, such as improving health status, protecting health or reducing the risk of disease. It is a part of nutrition. It is not a tablet, capsule, pill, or other food supplement and cannot be used for this purpose [8]. In Canada, functional foods can be similar to any food in terms of external appearance. It can be consumed as part of a diet currently being carried out, and the scientific effects are indicated. In addition to its nutritional properties, it can also be used to minimize the risk of certain diseases [9]. According to the Institute of Food Technologies, foods or food ingredients supplying additional health benefits beyond normal nutrition [10]. The International Life Science Institute states that this particular food contains food components with physiological effects, which go beyond regular nutrition and offer additional health benefits [11]. As the International Food Information Council, a food or its components that contribute to improving health, reducing the risk of specific diseases and health conditions, and providing normal nutrition [12].

In the light of the Academy of Nutrition and Dietetics declarations, foods that contain foods that are defined as enriched, varied or enhanced foods beyond the nutritional features of foods are foods providing benefits on body health on condition of its consumption as part of a regular diet [13]. Food and Agriculture Organization declares that they are foods consumed being a part of a usual diet and include active ingredients in biological aspect to retain health and diminish the risk of disease [14].

There are some vital functional characteristics:

1. The medicine should not be capsule formed, tablets, or other food supplements.
2. It should have scientifically proven health effects.
3. Beyond its use as food, it should have some positive effects for health, such as promoting one or more well-being and/or minimizing the risk of disease,
4. It should be a continuation of normal nutrition,
5. The proportion of one or more of its ingredients should be increased,
6. The added food ingredient must have a positive health effect,
7. In scientific terms, the daily pattern of use and intake should be determined,
8. The functional food consumed must be proven to be safe,
9. The quantitative and qualitative characteristics of the food ingredient must be determined,
10. Functional food should be traditionally suitable for consumption,
11. Functional food should never be a product used as a medicine,
12. These products are part of a normal diet [15].

Functional ingredients in foods

1. β -carotenes
2. Sugar alcohols

3. Vitamin and minerals
4. Dietary fibers
5. Oligosaccharides
6. Antioxidants
7. Phenols
8. Lactobacteria
9. Unsaturated fatty acids
10. Antioxidants
11. Glycosides, isoprenoids
12. Peptides and proteins
13. Prebiotic and probiotics
14. Conjugated Linoleic acids

In short, the benefits of functional food consumption include improving cardiovascular health, reducing oncological risks, reducing the risk of diabetes and blood pressure, improving sensory properties, strengthening the body's immune mechanism, delaying aging and protecting the body from some related disorders, improving problems such as obesity and weight gain, eliminating urological and gastrological disorders, playing a supportive role in bone and blood disorders, protecting the body from microbiological effects, etc. [16].

1.2. TYPES OF FUNCTIONAL FOODS AND HEALTH EFFECTS

Since the term functional food includes foods including some benefit for health along with nutrients, it is seen as foods that not only meet the basic nutritional requirements of the body, but also help individuals to be healthier and protect them from various diseases by providing additional benefits to metabolic activities and physiology [17]. Research on the fact that nutritional deficiencies cause various health problems and that these problems can be

prevented by taking the necessary food components has been an important area of nutritional science throughout history. Defining basic nutrient requirements to avoid nutritional deficiencies was the main focus of nutrition science between 1900 and 1950. This has led to the establishment of reference nutrient intakes for vitamins, minerals, proteins, fats, carbohydrates and energy, and reference for the population and reference nutrient values. The cornerstone of a healthy diet is to create a balanced diet that balances physical and metabolic activities and energy intake and provides the necessary nutrient intake. This has made it possible to better understand the possible beneficial and harmful effects of dietary nutrients and other ingredients, and to produce specific specialty foods that may affect physiological and metabolic functions other than the basic requirements of nutrition. These foods are considered “functional” [18]. According to scientific research, some meals and dietary components have biological effects on the body and may provide particular health advantages. Functional foods often have positive health impacts because of the bioactive substances they contain. Various substances have different impacts on the body, and they often interact to change one or more physiological processes. Consuming foods in their natural state as much as possible is vital in order to get the health advantages of the numerous chemicals found in diverse foods [19]. Functional foods can consist of various types, bio-active components, and active ingredients. Each of these has different health impacts (Table 1.1).

Table 1.1. Types of functional foods and their health effects

Health Effect	Sample type
It has positive effects on gastrointestinal health, immune function, and cancer	Lactic acid bacteria and bifidobacteria
It selectively increases the growth and activity of one or a limited number of bacteria in the colon.	Fructo-oligosaccharides, inulin, isomalto-oligosaccharides
It reduces cholesterol levels, stimulates antioxidant function, prevents stunted or retarded growth and development and deformation of bones	Drinks containing vitamins A, C, E, and cholesterol-lowering drinks
It helps the growth of probiotic microorganisms	Oats, barley, rye
It reformulates fatty acid profiles. Also, it incorporates antioxidants, dietary fiber and probiotics into the body's working system.	Fermented meat products
It helps to reduce the formation of possible blood clots. It provides control of blood pressure.	Eggs enriched with omega-3.

It's critical for foods to include bioactive substances. Health is affected by potential impacts on digestion, absorption, and metabolism, as well as by the accompanying meals, drinks, and nutrients ingested, oral and intestinal microbes, intake volume, and duration. The quantity and kinds of bioactive chemicals present in food when ingested may also be influenced by elements in the production of food chain, to illustrate; food processing and distribution (Table 1.2) [19].

Table 1.2. Examples of functional foods, bioactive food components and health effects

Food	Bioactive Food Component / Class	Potential Effects on Health
Tomato	Carotenoids (lycopene, beta-carotene, etc.), vitamins C and A and potassium	Supports cardiovascular health, may reduce the risk of certain cancers such as mouth, pharynx, larynx, prostate and lung cancer
Blueberry	Flavonoids (anthocyanins, phenolic acids, etc.), pterostilbene, vitamins C and K, manganese and dietary fiber	Supports cardiovascular, metabolic and brain health and may reduce the risk of cancers such as mouth, pharynx, larynx and lung cancer
Fermented Milk/Milk products	Bioactive peptides, probiotics, calcium and protein	Supports intestinal, immune, cardiovascular and metabolic health
Walnut	Ellagitannins, phenolic acids, omega-3 fatty acids (alpha-linolenic acid), phytosterols, melatonin, vitamin E, copper, manganese and magnesium	Supports cardiovascular and brain health and may reduce the risk of certain cancers such as breast and prostate cancer
Broccoli and cruciferous vegetables	Glucosinolates, vitamins C and K, manganese, folate, fiber	May reduce the risk of certain cancers such as colorectal, oral, pharyngeal, laryngeal and lung cancer, cardio-protective, improve antioxidant defenses
Salmon and other oily fish	Omega-3 fatty acids	Supports eye, brain, and cardiovascular health
Whole grains	Flavonoids, saponins, lignans, resistant starch, B vitamins, vitamin E, selenium, manganese, dietary fiber and protein	Supports intestinal and cardiovascular health and may reduce the risk of colorectal cancer

1.3. INTENDED USE OF FUNCTIONAL FOODS

The possibility that functional foods, like many other foods, may have negative effects, should not be ignored. These positive effects may turn into negativity in excessive doses of some components that are determined to have positive effects. The side effects of functional foods and products are evaluated as in other foods, and it is stated that the negative effects that may occur with the intake of these foods may be caused by the natural components of the food or the functional food ingredients added to the food to provide functionality. The most common of these reactions are toxic and non-toxic effects that vary based on the sort of ingredient and the quantity of intake, and food components that cause food allergies and intolerance. Products that are labeled “natural” and provide herbal support do not necessarily mean that they are completely safe or free of possible side effects. Depending on the excessive use of functional foodstuffs with physiological activities, it is essential to emphasize and examine the conditions that may pose risks together with the benefits on individual and public health carefully and seriously. Many studies emphasize the positive and recovery effects of these foods on health [20]. Advances in scientific technology have developed our comprehension of the relationship between nutrition and disease, and the role of functional foods in protecting and improving our health has attracted more attention. Foods are now considered beyond just the energy and a few nutrients they contain. This has been influenced by the awareness of consumers and the change in consumer demands, along with the effectiveness of scientific technology research. In particular, people in developed countries such as Japan and America have changed the expectations of their daily diets. They determine their daily diet by carefully selecting every food they take, thinking about the benefits it will bring to themselves and their health, rather than just being fed or just eating [20].

The following reasons have led consumers to raise awareness and change their daily diets [1]:

- Rapid developments in science and technology
- Increase in treatment prices
- Increase in the elderly population

- Changes in lifestyle
- Consumer demand for quality and variety
- Raising public awareness regarding the relationship between food and health
- Changes in the food marketing system

All these reasons have led consumers to search for different diets and food companies and research and development departments to seek answers to consumer demands. As a result, functional foods have gained importance.

A functional food is either the food itself or a food obtained by using functional food ingredients. Functional foods according to their production and intended use are stated as follows [21]:

- Medicinal foods
- Dietary supplements (food supplements)
- Special dietary foods
- Enriched foods
- Nutraceuticals.

1.3.1. Special Dietary Foods

Foods formed and adapted to provide dietary requirements concerning physiological and physical cases and/or a variety of health problems. The formulation of those foodstuffs significantly should not be same as normal foodstuffs. Infant formulas, reduced energy or low energy foods to control weight, foods for special medical purposes, low sodium or sodium-free salts and low sodium foods, sports foods, diabetic foods can be stated as the examples of such foodstuff [22].

1.3.2. Medicinal Foods

According to the Codex Alimentarius, it is defined as a “category of food with a special dietetic purpose, specially produced or formulated and intended for use in patient nutrition and only under the supervision of a doctor”. According to this definition, two specific characteristics attracts the attention [22].

- Targeting patients
- Necessity of doctor supervision

1.3.3. Dietary Supplements (Food Supplements)

According to the Dietary Supplement Health and Education Act (DSHEA), which started to give service in the USA in 1994, the phenomenon “food supplement” refers to “herbal drugs, vitamins, minerals, amino acids, organ tissues, enzymes, glands and metabolites chained to foods for oral ingestion”. Extracts and concentrates are also included in this term. They are available as tablets, capsules, soft gel, gelatin capsules, liquid or powder [23]. Therefore, they cannot replace the daily diet and cannot be consumed as a normal foodstuff within a meal [23].

1.3.4. Enriched Foods

According to Codex Alimentarius, enrichment can be added to one or more required nutrients for a food, naturally or not in its content, in order to compensate for a proven deficiency in a population or a specific group of the population [24].

1.3.5. Nutraceuticals

In general, the definition of nutraceutical food can be used synonymously with functional foods and includes processed products such as isolated nutrients, nutritional supplements,

“designed” foods, functional foods, herbal products, cereals, soups and beverages [25]. Nutraceuticals provide physiologic benefits and protection against chronic diseases [25].

1.4. OYSTER MUSHROOM AS A FUNCTIONAL FOOD

Over 2,000 different kinds of mushrooms may be found in nature, but only nearly 25 of them are commonly consumed as food and just a small number are raised for commercial purposes [26]. Yet, it may be difficult to distinguish between edible and medicinal mushrooms since various of popular food species offer medicinal benefits and numerous medicinal mushrooms are also edible. *Agaricus bisporus* is the fungus that is most often farmed, and successively *Lentinus edodes*, *Pleurotus* species, and *Flammulina velutipes*. Mushroom production is constantly rising, and China is regarded the world's widest producer currently. Nonetheless, the nutritional, sensory, and specifically pharmacological qualities of wild mushrooms are causing them highly vital [27]. Eating mushrooms contains a lot of carbohydrates, such as chitin, glycogen, trehalose, and mannitol, as well as fiber, β -glucans, hemicelluloses, and pectic materials. Moreover, produced edible mushrooms contain large levels of the sugars glucose, mannitol, and trehalose, but only modest amounts of fructose and sucrose. They offer exceptional nutritional value since they are low in fat but high in essential fatty acids, relatively rich in protein, and include high amount of fiber and necessary amino acids [28].

P. pulmonarius sp. frequently referred to as "oyster mushrooms." The *Pleurotus* genus has roughly 40 species [28]. These mushrooms produce fruiting bodies with a shell-like structure that are abundant in protein, vitamins, and minerals and own a variety of lignocellulosic substrates on which they may develop [29]. Some of them include healthy ingredients like taurine and β -glucan in them [30]. Throughout the whole globe, they are the second well-known mushroom following the button mushroom, and they are particularly well-liked in Bangladesh. Nowadays, oyster mushrooms are professionally farmed all over the globe for food. They were initially cultivated in Germany with the aim of survival during World War II. Aspartate transaminase (AST) and Alanine transaminase (ALT) are hepatic enzymes that are dramatically decreased by oyster mushrooms in plasma [31]. High-molecular weight polysaccharides, such as β -glucans, peptides, and proteins, make up the majority of bioactive

substances [32]. The glucan molecules in oysters help the body's immune system fight off aberrant cells and strengthen it against the negative effects of chemotherapy and radiation treatments used to eradicate tumor cells. Folklore claims that *Pleurotus* may also prolong life, lower blood pressure, and help individuals recuperate from tiredness. It may also avoid obesity, constipation, and hangovers. A few examples of bioactive substances involve lectins, which have immunomodulatory, antiproliferative, and antitumor properties; phenolic compounds, involving antioxidant ingredients; and polysaccharides (polysaccharide proteins and polysaccharide peptides), with immunostimulatory and anticancer properties. The edible oyster mushroom may also qualify as a functional food since it has anti-inflammatory properties [33]. In addition, *P. pulmonarius* sp. demonstrates a hypocholesterolemic impact in rats with normal cholesterol levels, hypercholesterolemia, or inherited cholesterol abnormalities. *P. pulmonarius* spp. has shown an anti-inflammatory effect in rats with colitis and is reportedly the greatest source of fungal β -glucans. Conversely, *P. pulmonarius* sp. is a possible source of anti-inflammatory and cancer-preventing chemicals [32,33]. In terms of yield, texture, taste, and shelf life, this mushroom is outstanding. This mushroom contains folic acid, which is used to treat anemia. This mushroom has anti-diabetic properties and is high in antioxidants. There have been reports on the purification and characterisation of laccase enzyme as well as its function in the decolorization of various dyes [34]. Due to a number of variables, it has been difficult to identify edible mushrooms, especially those of the *Pleurotus* genus, up until this point. The *Pleurotus* genus contains cultivated edible mushrooms that may be identified mostly by their physical characteristics, such as the length, thickness, and color of the stipe and the form, color, and size of the hymenophore [34].

1.5. β -GLUCAN

The FDA has determined that in order to lower total blood cholesterol levels, and it is suggested to have a daily minimum intake of 3 g of β -glucans. The health statement simply mentions the required amount of oat β -glucans; however, it makes no mention of the physicochemical characteristics, including solubility and molecular weight, lately been shown to be crucial for their physiological effects [35,36]. The most important chemical compounds in contemporary medicine are polysaccharides, and β -glucan is the most popular

and changeable metabolite with a broad range of biological action. A soluble fiber called β -glucan is generated from the cell walls of plants, bacteria, fungus, yeast, and algae. The structure, solubility in water, molecular weight, and size of β -glucans derived from mushrooms vary. They display a variety of health-improving advantages [37,38]. Although β -glucans in grains (such as oats and barley) include 1, 3 and 1, 4 links, those in yeast and mushrooms possess 1, 3 β -glucan connections and sometimes 1, 6 β -glucan connections [39]. The β -1,3/1,6 glucan produced from yeast is said to have more biological activity than its 1,3/1,4 bounds. The solubility of β -glucans affects their efficacy [40]. Oyster mushrooms are one of the most significant resources of β -glucans, claim Rop *et al.* (2009). Plant hemicellulose polysaccharides (soluble fibers) known as β -glucans are understood to be hypocholesterolemic substances [41,42]. It has been shown that those with hyperlipidemia may see a drop in blood cholesterol of up to 7.5%. The physiological benefits of β -glucans and a number of other viscous plant polysaccharides, such as guar, pectin, are often related to a decline in postprandial blood glucose levels. The ability of this polymer to generate an unstimulated water layer has been linked to this effect. By preventing the convective effects of intestinal contractions, this layer prevents the small intestine from absorbing as much sugar. Foods that are known to collect significant levels of β -glucans, such as oats, barley, beans, and gums, have also been linked to this impact [41,42]. Since locust β -glucan increases the viscosity of the dietary ingredients in the digestive tract, it lowers blood glucose levels [43]. The β -glucan substance lowers blood levels of bad cholesterol by creating a gel-as network, trapping bile acids, and excreting them from the colon. Owing to its thickening flow behavior in the aqueous phase, β -glucan has been used as a hydrocolloid in various experiments. β -glucan may be employed as a stabilizing element in various dishes, like sauces, salad dressings, and ice cream, due to its high viscosity. Investigating the flow dynamics and stabilizing effects of β -glucan obtained from various sources is thus required. According to Wood *et al.* (1977), the viscosity of β -glucan is reduced when shear force is applied to the β -glucan solution during the extraction and purification operations at pilot plants as opposed to on a laboratory scale [44].

1.6. DIETARY FIBER

A broad variety of substances, principally carbohydrates, which can not digested in the small intestine are collectively referred to as "dietary fiber" [45]. Cellulose, hemicellulose, pectin, guar gum, psyllium, and dextrin are the many fiber kinds. It is commonly accepted that fibers may have a variety of physiological impacts on people, such as a shorter intestinal transit time, more bulk in the stool, better control overweight, lower blood cholesterol levels, and lower post-meal blood sugar and insulin levels. Nevertheless, not all of the substances referred to as "dietary fiber" alone are equally beneficial [46]. Consumers are placing a greater emphasis on eating wholesome, low calorie, nutritionally balanced meals that include dietary fiber (DFs). DFs have garnered attention for their positive impact on general health for some time. Depending on their DF content, a wide variety of culinary applications are being supplemented and promoted. DFs have been studied for their benefits in the management of serum cholesterol levels, the prevention of diabetes and intestinal cancer, the treatment and prevention of constipation, and the promotion of good microbes [47]. According to their many functional characteristics, including the fiber source, type, and degree of processing, different DFs may be used for culinary applications to varying degrees [48]. Soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) are the two main groups of DFs [49]. Soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) are known as being two main groups of DFs [50]. Yet, numerous definitions of fiber exist among scientific and governmental organizations worldwide. The Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU) set up a legally accepted definition of DF in 2009 [51]. If carbohydrate polymer consists of ten or more monomeric units and cannot be broken down by the enzymes in the human small intestine, it is classified as dietary fiber. This classification is based on its inclusion in one of three specific categories: (1) Naturally occurring edible carbohydrate polymers that are consumed with food. (2) Carbohydrate polymers that have been made from dietary sources through physical, enzymatic, or chemical processes and have been illustrated to competent authorities to include physiological effects that are beneficial to health. (3) Synthetic carbohydrate polymers that have been scientifically researched and demonstrated to possess physiological effects beneficial to health. Source, chemical qualities, resistance to digestion, and advantageous physiological consequences are the four ways that fiber has been described by scientists [52].

Biological classifications defining the origin of fiber have conventionally included non-starch polysaccharides originating from plant cell walls. Another explanation from the beginning presents a sample: "DF is the proportion of food generated from plant cellular walls, digested with excessive amount poorly in people bodies" (52). The structural variations between SDF and IDF have various physiological effects [53]. SDFs have been associated to the reducing of blood cholesterol levels and the decrease in the intestinal absorption of glucose, while IDFs are related to the absorption of water and regulating intestinal effects [54]. These diverse physiological results are mostly dependent on the anatomical and physical characteristics of a certain kind of DF. These variances result in dietary fibers (DFs) showcasing a variety of in vivo characteristics such as hydration, swelling, and susceptibility to enzymatic degradation [55]. Cui *et al.* [56] have thoroughly examined the correlation between the chemical structure, molecular weight, and relevant physiological properties of dietary fibers (DFs).

DFs may help to regulate digestion because of their effect on the rate of starch degradation and their capacity to inhibit excessive glucose absorption. When DFs are added to meals that include starch, the passage of water is restricted while starch granules may be retained. The result is a limitation on the digestive enzymes' access to the starch granules, which reduces the pGI [57].

1.6.1. Soluble Dietary Fiber (SDF)

Soluble dietary fiber (SDF) is specifically defined as dietary fiber that has the ability to dissolve in aqueous enzyme and buffer solutions, which mimic the conditions found in the human digestive system [58]. Non-viscous soluble fibers serve largely as a substrate for microbial fermentation in the colon, while SDFs delay stomach emptying and reduce glucose absorption, lengthening the overall transit time [58]. SDFs are composed of oligosaccharides such as fructooligosaccharide (FOS), pectins, β -glucans (found in mushrooms, oat and barley grains), galactomannan gums, alginate, and psyllium. The term "fructans" refers to the group of fructooligosaccharides (FOS), which also go by the names oligofructose and inulin [59]. Most main cell walls include pectin, which is especially prevalent in the non-woody portions of terrestrial plants. The pectin highly exists in the fruit coat, nevertheless it is also seen in

trace levels in a different sort of fruits and vegetables, including apples, pears, apricots, cherries, and oranges. Pectin is known to be a linear polysaccharide that mostly consists of 300 to 1000 monosaccharide units of D-galacturonic acid [60,61]. The polysaccharides comprising D-glucose monomers connected by glycosidic linkages are known as β -glucans. They are most often found as cellulose in plants, cereal grain bran, baker's yeast cell walls, and certain fungi, mushrooms, and bacteria [62]. Due to their strong ability to bind water as well as their emulsification and viscosity-increasing capabilities, galactomannans (GMs), polysaccharides containing a mannose backbone and galactose side groups, are often utilized in foods as stabilizers [63]. Alginates are unbranched polysaccharides made of D-mannuronic acid and -guluronic acid that are 1–4 connected (64). Alginates, which are uniquely found in algae, are extensively dispersed in the algae cell walls and are also an exopolysaccharide of bacteria, such as *Pseudomonas aeruginosa* [64]. They bond to water to generate viscous hydrogels that may be used as thickening agents and in a variety of biological applications (64). Psyllium is the common name for a number of *Plantago* species, whose seeds are utilized in the manufacture of mucilage on a commercial scale [64]. The seed, the seed husk, and the whole plant are all referred to as "psyllium." Psyllium is grown because its seed husk, at the same time referred as psyllium hydrophilic mucilloid, psyllium hydrocolloid, and psyllium seed gum, is a rich source of SDF [65]. According to certain research [66], SDF prevents type II diabetes because of the viscosity of the soluble fibers.

1.6.2. Insoluble Dietary Fiber (IDF)

Resistant starch, lignin, cellulose, and a few hemicelluloses make up the majority of IDFs. Cellulose, a polysaccharide consisting of a linear chain of several hundred to tens of thousands of correlated D-glucose units, is the most abundant organic polymers on Earth [67]. It makes up around 25% of the fiber in grains and fruit and about a third of the fiber in vegetables and nuts, and it is the main ingredients of the cell walls of the majority of plants [67]. Cellulose makes up a significant portion of the fiber in grain bran. In addition to glucose, hemicelluloses are polysaccharides that also include other carbohydrates. They are both soluble and insoluble in water and are connected to cellulose in cell walls. Hemicellulose makes up around one-third of the fiber found in fruits, vegetables, legumes, and nuts. Cereal grains are the principal dietary sources of hemicellulose [68]. After 120

minutes of intake, the resistant starch (RS) portion of the starch ferments in the colon but is not converted to D-glucose by amylase in the small intestine [61]. Whole grains, cooked and cold pasta, legumes, rice, potatoes, and unripe bananas all contain resistant starch [61]. There are three main subtypes of RS: RS1, RS2, and RS3. Starches that are physically difficult to reach, such as those found in whole or partly milled grains or seeds, are referred to be RS1. Granules of raw starch, such as those seen in high-amylose maize, banana, and potato starches, are referred to as RS2. Retrograded starches are referred to as RS3 and include those produced from unaltered starch or as a consequence of food processing applications. The most common source of lignin, a complex polymer of aromatic alcohols, is wood. It is necessary for filling the gaps in the cellulose, hemicellulose, and pectin-based secondary cell walls of plants [61]. Excellent sources of lignin include foods like celery and the outermost layers of cereal grains that include a woody component. IDFs often have a laxative effect by decreasing the intestinal transition time, increasing fecal volume, and excreting bile acids [61].

1.6.3. Total Dietary Fiber (TDF)

Soluble and insoluble DF in different levels are present in almost all naturally occurring high-fiber diets [68]. Cereal DF is mostly derived from whole grains and bran products, while SDF is primarily derived from fruits, vegetables, and mushroom, oat and barley products (soluble β -glucans) [69]. There is a significant relationship between whole grain and cereal DF consumption and the amount of TDF found in whole grain food items, which is about 12% [68]. Whole grains are cereal grains that include the cereal germ, endosperm, and bran, as opposed to processed grains that only sustain the endosperm. Wheat, oats, and barley are examples of typical whole grains [68]. Many breads and cereals, which are made with bran (the tough outer layers of cereal grains), involve up to 25% TDF [68]. The herbal components employed in the formulations contributed to the dietary fiber content. Herbs and spices are being used to enhance the sensory qualities and enhance the shelf life of food since the dawn of civilization. As a result, food producers have been highly inspired to create a variety of fiber-enriched or fiber-fortified food items as a result of the growing public knowledge of dietary fiber's potential health advantages [68,69].

1.6.4. Structure of β - Glucan

The form of linear (1 $\rightarrow$ 3)- β -glucan, the basic molecular structure of β -glucan is as shown in Figure 1.1.

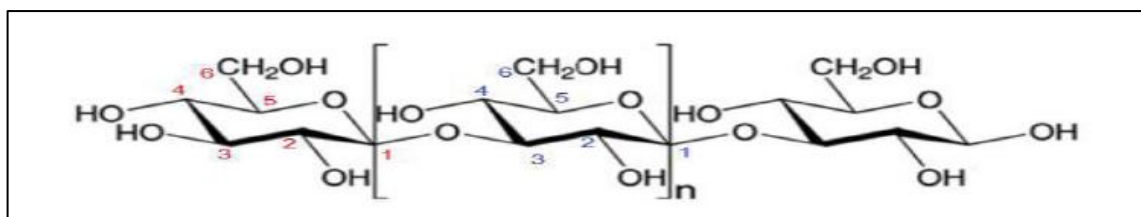


Figure 1.1. Structure of linear (1 $\rightarrow$ 3)- β -glucan

The molecular structure of β -glucan varies as to source from which it is obtained [70]. The branching structures of β -glucan are divided into three basic classes as shown in Figures 1.2, 1.3, and 1.4.

a) (1 $\rightarrow$ 3) (1 $\rightarrow$ 2)- β -Glucan

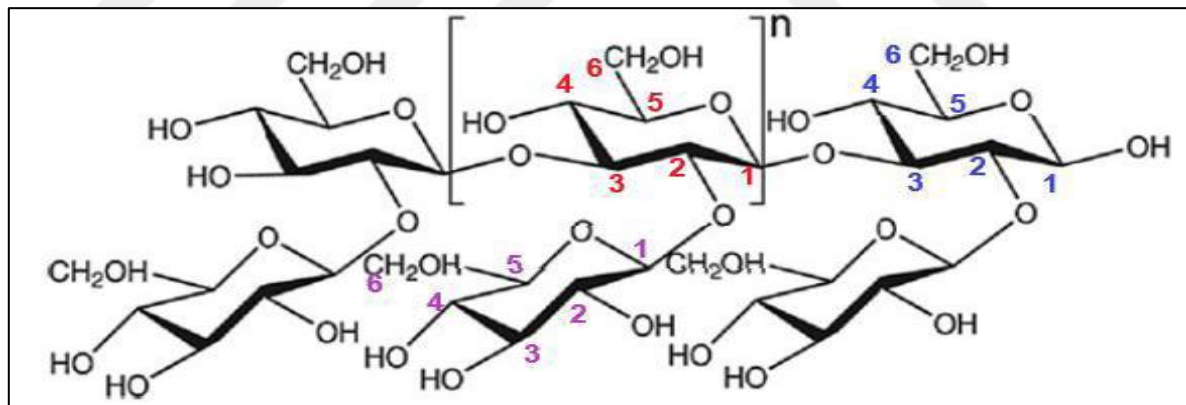


Figure 1.2. Structure of (1 $\rightarrow$ 3) (1 $\rightarrow$ 2)- β -glucan

The capsule of type 37 *Streptococcus pneumoniae* species was only homopolysaccharide. There are 90 types of pneumococcal capsules. This capsule type is one of two neutral polysaccharides. The glucan in this capsule is dissolved in water and DMSO [70].

b) (1 $\rightarrow$ 3) (1 $\rightarrow$ 4)- β -Glucan

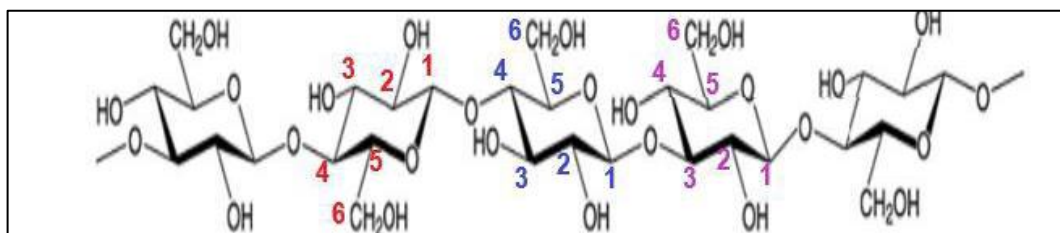


Figure 1.3. Structure of (1→3) (1→4)-β-D-glucan

(1-3) (1-4)- β-D-glucans exist in cereals (barley, oats and wheat), grasses, agrimony, mosses, mushroom (chromistanes and capped), algae (green and sulfated red) [70]. Such β-glucans can usually be solved in the water.

c) (1→3) (1→6)-β-Glukan

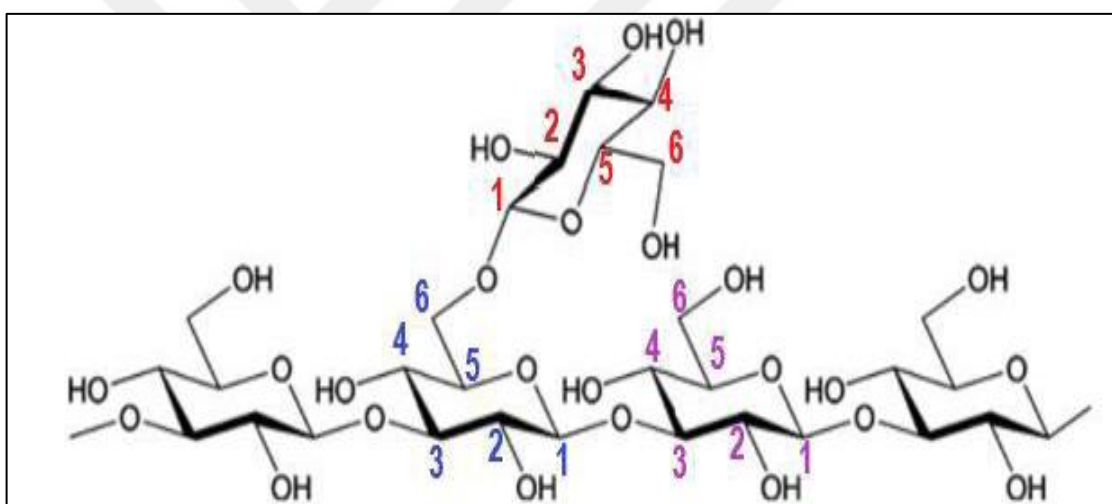


Figure 1.4. Structure of (1→3) (1→6)-β-D-glucan

(1→3)(1→6)-β-D-glucans exist in branched and cyclic forms. Branched (1→3)(1→6)-β-D-glucans are found in yeasts, mushrooms, brown algae, diatoms, ascomycetes, and basidiomycetes. Cyclic (1→3) (1→6)-β-D-glucans are seen in *Bradyrhizobium japonicum*, *Rhizobium loti*, *Azospirillum brasilense*, and *Azorhizobium caulinodas*. These microorganisms live symbiotically in legumes [70]. The features of β-glucan varies in the source accordingly where it is obtained. The reason is that different molecular structure include β-glucans from various sources.

1.7. EFFECTS OF PHYSICAL AND CHEMICAL PROPERTIES ON THE BIOLOGICAL ACTIVITY OF β -GLUCAN

The physicochemical properties of β -glucan are changeable regarding the area of use. The solubility of β -glucan is significant for application areas. Especially there is a relationship between its water solubility and usage in food and health. In these areas, it is desirable that β -glucan is water-soluble. Molecular mass, degree of polymerization, and molecular structure affect its solubility. As the molecular mass of β -glucan increases, its solubility decreases [71]. The degree of polymerization is the average number of repeated units per polymer chain [72]. If the degree of polymerization is accepted higher than 100, β -glucan cannot be solved in water [73]. In the literature, the molecular structure of β -glucan are divided into three classes according to their solubility. These are (1-3)- β -glucans insoluble in alkali and acetic acid, (1-3)- β -glucan soluble in alkali, and branched in a wide range (1-6)- β -glucan [74]. These three factors are correlated with each other. As the molecular mass of β -glucan and therefore the degree of polymerization increases, the β -glucan chain becomes a ball and the molecular structure becomes complex. In this case, it cannot dissolve.

The molecular masses, structures and viscosities of glucans vary according to the source from which they are obtained. As the molecular mass increases, viscosity increases and solubility decreases. This situation affects both the biological activity and usage areas of β -glucan. Many studies aim to show that the molecular mass of β -glucan affects the biological activity. Although β -glucans with a molecular mass of 100-200 kDa show the best anti-tumor activity, β -glucans along with a molecular mass of 5-10 kDa either did not show anti-tumor activity or showed limited anti-tumor activity [74]. Molecular mass affects physicochemical properties such as viscosity, foaming capacity, solubility, oil binding capacity, swelling power as well as biological activities and its usage area changes according to these properties. It is necessary to know the molecular mass of β -glucan is increasing. To illustrate, as high-molecular-mass water-insoluble β -glucans are used as prebiotics, lower-molecular-mass water-soluble β -glucans are utilized as food additives in foods [75,76]. Foaming capacity and stability are an important property for materials used in foods. This property is prominent as they are used as food additives and stabilizers in foods. When added to cakes and doughs, it provides high foaming and increases the consistency. A negative correlation is present between viscosity and foaming capacity. Materials with higher viscosity have less

foaming [77]. As the molecular mass increases, viscosity increases with decreasing solubility. Water binding capacity is known as the quantity of water left in the fiber followed by applying an external force [77]. As the molecular mass increases, viscosity increases with decreasing solubility. Water binding capacity is the amount of water remaining in the fiber after applying an external force [77]. Water binding capacity of β -glucan is high. It shows that it is possible to use that to solve the water separation problem in various products such as jam and cheese [77]. Free radicals, that are defined as molecules containing one or more unpaired electrons, are high-energy and unstable. Free radicals can be sulfur, carbon, hydrogen and nitrogen centered. Antioxidants pair their electrons with free radicals, making them more stable and harmless. As to results found in the studies, β -glucans showed antioxidant activity and especially β -glucan obtained from wheat showed higher antioxidant activity than polymers such as pectin and chitosan [78].

Moreover, as the molecular mass increases with the structural complexity of β -glucan, its effect on immunity and antitumor activity increases.

Long-chain β -glucans are attached to macrophages by macrophage dectin-1 receptors. In macrophages, β -glucans are broken down into particles. β -glucan particles attach to granulocytes via complements (CR3). Monoclonal tumor cells with complement (iC3b) in the tumor cell opsonize tumor cells. The opsonized tumor cell is destroyed by phagocytosis via granulocytes. The role of β -glucan is to stimulate the immune cells and direct them to the tumor cell [79]. Dectin-1 is a lectin acceptor and binds to β -glucans [80]. Lectins are carbohydrate-binding glycoproteins or proteins. Macrophages are a type of white blood cell. In the innate immune system, macrophages are one of the two cells with phagocytic function. These cells have the capacity to get, identify, and destroy pathogens. Opsonin are the substances such as antibodies or complements that bind to bacteria or antigens and facilitate the phagocytosis of antigens by macrophages and neutrophils. Opsonin are the substances such as antibodies or complements that bind to bacteria or antigens and facilitate the phagocytosis of antigens by macrophages and neutrophils. Opsonize means sensitizing a certain antigen to phagocytosis with the help of opsonin. Monoclonal antibodies are those that bind to only one type of epitope (binding site) on antigens. The antimicrobial activity of β -glucan is similar to its anti-tumor activity. The only difference is that cells such as viruses, bacteria and parasitic fungi are destroyed instead of tumor cells. The role of β -glucan

is to stimulate immune cells and direct them against viruses, bacteria, parasites, and fungal cells. According to results found in the studies, β -glucan has a good antimicrobial activity against different types of microbes [81].

1.8. β -GLUCAN SOURCES

β -glucan is derived from three sources which are cereals, mushrooms, and microorganisms. Its properties and molecular mass vary according to the sources from which it is obtained. Considering the parts of the sources, β -glucan was generally seen in the cell walls of the sources (Table 1.3)

Table 1.3. Different sources of β -glucan, their chemical structures and locations [70, 82]

Chemical structure	Source	Current Location	Reference
(1 $\rightarrow$ 3)- β -glucan	<i>Pleurotus sajor-caju</i>	Cell wall	83
	<i>Rhodotorula mucilaginosa</i>	Cell wall	84
(1 $\rightarrow$ 3)(1 $\rightarrow$ 4)- β -glucan	Wheat (<i>Triticum vulgare</i>)	Endosperm cell wall	85
	Oat (<i>Avena sativa</i>)	Endosperm cell wall	86
	Barley (<i>Hordeum vulgare</i>)	Endosperm cell wall	87
(1 $\rightarrow$ 6)- α -glucan	<i>Ipomoea batatas</i>	Root	88
(1 $\rightarrow$ 4)- α -glucan	<i>Lonicera japonica</i>	Extracellular	89
(1 $\rightarrow$ 6)- β -glucan	<i>Agaricus bisporus</i>	Extracellular	90
(1 $\rightarrow$ 3) (1 $\rightarrow$ 6)- β -glucan	<i>Schizophyllum commune</i>	Cell wall	91
	<i>Lentinus edodes</i>	Cell wall	88
	<i>Saccharomyces cerevisiae</i>	Cell wall	92
	<i>Candida albicans</i>	Cell wall	93
	<i>Aspergillus fumigatus</i>		

β -glucans obtained from different sources show different biological activities (Table 1.4).

Table 1.4. Structures, sources, biological effects of β -glucan [94]

Structure	Source	Effects
β (1 $\rightarrow$ 3)(1 $\rightarrow$ 6)	<i>Saccharomyces cerevisiae</i>	Anti-parasitic
		Anti-bacterial

		Anti-viral
		Anti- fungal
		Anti-mutagenic
		Anti-tumor
		Mitogenic
		Hematopoetic stimulant
		Antioxidant
	<i>Candida albicans</i>	Immunostimulation activity
	<i>Poria cocos</i>	Anti-tumor
	<i>Agaricus blazei</i>	Cytokine induction
		Anti-tumor
		Anti-mutagenic
		Inhibition of CYP450 isoenzymes
	<i>Lentinus edodes</i>	Anti-tumor
		Inhibition of CYP450 isoenzymes
	<i>Schizophyllum commune</i>	Anti-tumor
	<i>Coriolus versicolor</i>	Anti-tumor
$\beta(1\rightarrow3)$ (1 $\rightarrow$ 4)	Oat	Anti-microbial
		Anti-parasitic
		Hypocholesterolemic
		Anti-thrombotic
	Barley	Anti-mutagenic

1.9. USE OF β -GLUCAN AND ITS HEALTH EFFECTS

If fat is replaced with different food combinations, it can be beneficial for the health through preparations varied in β -glucan. It is also related to intakes of lower fat and energy. At the same time, mushroom β -glucans illustrate that all the physiological properties attributed to dietary fiber (DF). Recent epidemiological data suggests that diets rich in dietary fiber extracted from whole cereal grains and mushrooms are associated with lower risks of type II diabetes, obesity, mortality, incident cardiovascular diseases, and ischemic stroke. This correlation is attributed to a range of metabolic processes, including reductions in plasma cholesterol, blood lipids and blood pressure, as well as improvements in insulin sensitivity and blood glucose control [95].

Even small reductions may be significant when seen in the context of the whole hypercholesterolemic population since a 1% reduction in blood cholesterol might result in a 2% reduction in heart disease mortality. In theory, soluble fibers reduce the incidence of coronary heart disease (CHD) by 20 to 40% by lowering blood cholesterol by 10 to 20%. Whole-grain consumption was shown to be connected to a 26% lower risk of CHD.

Improved glucose tolerance and decreased insulin requirements from cereal whole grains enriched in soluble DF provide further health advantages for type II diabetes patients as well as postponing or lowering the chance of acquiring the condition [96]. These nondigestible polymeric carbohydrates' physicochemical characteristics, such as their water-holding capacity, swelling, ability to prevent diffusion (via viscosity or gel formation), binding abilities, and vulnerable to bacterial degradation and fermentation, are thought to be responsible for several physiological functions of DF. Considering these finding, it is believed that dietary fiber (DF) has an impact on various aspects, including nutrient absorption, enterohepatic sterol metabolism, regulation of intestinal microflora, fermentation, and decomposition within the gastrointestinal tract. Furthermore, DF is thought to modulate digestive tract motility, gastrointestinal transit time, and the bulk and weight of stools [97]. They also have additional metabolic benefits, including lowering serum cholesterol and postprandial blood glucose levels. Insoluble fiber improves how the large intestine operates and alters situations for gastrointestinal function widely by decreasing intestinal transition time, raising fecal volume, delaying glucose absorption, and reducing starch hydrolysis. Nevertheless, these activities are not caused by a reduction in blood cholesterol or glucose levels [98].

1.10. DIABETES

1.10.1. Definition and Classification of Diabetes

DM is a bunch of diseases featured by elevated blood glucose and abnormalities in carbohydrate, protein, and fat metabolism because of the defects either in insulin secretion and insulin activity or both [99]. Insulin, which is secreted from the β -cells of the pancreas, which is necessary for energy in the body as well as helping to store excess nutrients, is not produced in patients with DM or the body does not respond to insulin. Elevated blood glucose due to ineffectiveness and/or insufficient insulin causes short-term and long-term complications in individuals with diabetes. Early signs of hyperglycemia are polydipsia (drinking too much water), polyuria (urinating too much), polyphagia (eating too much). Late complications involve vascular disease, peripheral neuropathy, and being prone to infection. The diagnosis is made through measurement of plasma glucose. Treatment

consists of medical nutrition therapy, exercise, and insulin and/or antihyperglycemic agents that reduce blood glucose levels. The prognosis depends on the degree of glucose control [100].

1.10.2. Type I

It usually starts under the age of 35 and account for 10% of all diabetics. Symptoms include excessive urination (polyuria), excessive thirst (polydipsia), spontaneous weakness, and fatigue. Children may also urinate in bed at night. People with this type of diabetes, caused by a sudden autoimmune disease, have destroyed the cells producing insulin in their pancreas. The defense system of the body destroys the insulin-producing β -cells. The result is either no or too little insulin secretion from the pancreas [99]. Without insulin, the body cannot use glucose for energy production, and since cells cannot absorb glucose, “blood glucose” levels increase. When the body cannot get the energy it needs from glucose, it starts to use fat for energy. As a result of the use of fats, acid residues called ketones begin to increase in the blood. This can lead to a serious problem called ketoacidosis. When people who have type 1 diabetes use sugar-reducing drugs that increase insulin delivery from the pancreas to the blood, they don’t have any effect. Type 1 diabetes can be treated with that the missing hormone insulin must be replaced from the outside. Insulin should be injected into the body as insulin is a protein and when taken orally, it is digested in the gastrointestinal tract and rendered ineffective. Insulin is only effective when injected under the skin [101].

1.10.3. Type II

People with this type of diabetes have some insulin secreted by their pancreas, however, it is either insufficient or ineffective. This is called insulin resistance. More than 90% of diabetes forms are type II diabetes. According to World Health Organization data, 90% of people with type II diabetes are seen as overweight or obese. The main problem in type II diabetes is known as the decreased beta cell function and insulin deficiency in peripheral tissues. Insufficient amount of insulin and resistance have a major part in the pathogenesis of this diabetes and hyperglycemia appears [102]. Chronic hyperglycemia causes long-term damage, dysfunction, or loss in various organs (especially eyes, kidney, nerves, heart, and

blood vessels). Due to the reason that hyperglycemia develops slowly, it may take some time to reach a severity that leads to the recognition of the classical symptoms of diabetes. Although the onset of type II diabetes is generally accepted to be in middle age or later, the incidence of type II diabetes has recently increased in children and adolescents [103]. Obesity is seen in approximately 55% of the cases with type II diabetes. In children and adolescents, increasing obesity in childhood has an important role in the development of type II diabetes. Heredity, obesity, pregnancy, long-term drug use, infections, physical and psychological traumas, and some pancreatic diseases are the basic reasons having an effect on the emergence of the disease. Although the pathogenesis of type II diabetes is not utterly comprehended, it is known that it is primarily caused by lifestyle and genetic factors. There are numerous lifestyle factors playing a role in the development of type II diabetes. Healthy diet, regular physical activity, keeping the body mass index at normal levels, and not smoking are the methods for changing lifestyle in order to prevent type II diabetes. The physiological effect of insulin differs in obese individuals with the comparison to normal-weight individuals. The reason of insulin resistance and hyperinsulinemia in obesity is particularly the reduced number of insulin receptors. Abdominal obesity is seen together with severe hyperinsulinemia and insulin resistance. Since obesity is frequently observed in individuals with type II diabetes, the insulin degree resistance increases. Among these individuals, the lipolytic activity of abdominal fat cells is quite dense and the antilipolytic activity of insulin is also more significant in abdominal fat cells. Inadequate insulin function leads to increased lipolysis, increased free fatty acids and impaired glucose oxidation in muscles, especially in the abdominal region in obese individuals [103]. When free fatty acid levels increase, it indicates decreased insulin clearance in the liver and consequently, decreased insulin sensitivity. In order to compensate this situation, blood insulin levels increase and thus, a vicious cycle of hyperinsulinemia-insulin resistance occurs. Hyperglycemia is very effective in the development of macrovascular complications; therefore, it is very important to ensure metabolic control in individuals with diabetes. In general, fasting plasma insulin levels are increased or normal in type II diabetes, and the insulin reaction to glucose tolerance is usually decreased. Resisting system for insulin is a general property in hepatic and peripheral tissues, such that receptor-related defects cause insulin resistance, and hepatic glucose uptake and glucose production are impaired as a result of oral glucose tolerance [102] .

1.11. GLYCEMIC INDEX (GI)

The concept of the glycemic index (GI) was first revealed by Canadian Professor Dr. David Jenkins in the 1980s. This refers to the rate at which a carbohydrate food raises blood glucose within a certain period of time after being eaten. The GI value of foods affects whether blood glucose rises slowly or rapidly. GI is a measurable value based on blood glucose responses after food consumption [104]. Blood glucose of a test food including 50 grams of carbohydrate along with a couple hours is found by comparing the area of increase with the increase in blood glucose produced by reference foods containing the same amount of carbohydrates. All carbohydrates have the different effects on blood glucose. Every food has a different effect on raising the blood glucose after meals. Some of them are absorbed more quickly from the intestines and raise blood glucose rapidly. According to the GI value, foods are classified into three groups as low, medium, and high GI foods. It is stated that if the GI is 55 and below, the Glycemic Index is low, if it is between 55 and 70, the Glycemic Index is moderate, and if it is above 70, the Glycemic Index is high [104].

High-GI meals cause reactive hypoglycemia by rapidly increasing blood glucose and insulin levels. This forms a basis for the formation of numerous chronic diseases in time. Low-GI foods diminish digestion and absorption of carbohydrates rates. Consequently, this reduces the need for reduced insulin and improves glycemic control and satiety. This has many health benefits in adults, including lower blood lipid levels and increased colonic fermentation in the patients with diabetes and hypertriglyceridemia [105].

1.11.1. Factors Affecting Glycemic Index

The GI of foods is influenced by the variables including food structure, the amount of protein, pulp and fat contained, particle size, cooking method, digestion, absorption rate and gastrointestinal motility differing among people. Among carbohydrates, monosaccharides are absorbed faster than polysaccharides, causing a sharp rise in blood sugar and rapid increases in insulin levels. Through consuming foods rich in fructose, glucose and sucrose, postprandial blood glucose is reached in a short time, and this is related to the high digestion and absorption rates of carbohydrates [106]. Glucose has a massive glycemic index

compared to fructose and sucrose. Thus, the glycemic index value is higher in foods with higher glucose content than sucrose and fructose [107]. A strong relationship has been found between glycemic index and amylaceous foods. Starch in maize, rice, wheat and potatoes consists of approximately 70-80% amylopectin and amylose. Amylose has a long chain structure that is difficult to be gelatinized during the cooking process. Amylose: the rate of amylopectin is the most effective factor having a role in cooking of rice and other amylaceous foods. Rice with high amylose content has a lower glycemic effect and lower GI. Foods with a higher rate of resistant starch have a lower glycemic index level. Some hemicelluloses, gums, β -glucan, pectin, water-soluble pulp slow down the intestinal passage of nutrients and glucose absorption, resulting in lower blood glucose levels. Insoluble pulp containing some hemicelluloses, lignin and cellulose slows starch hydrolysis and increases fecal volume, but it has limited effect on blood glucose levels. Foods with high pulp content, especially those rich in soluble pulp, prevent blood glucose levels from rising too much by reducing carbohydrate absorption. Foods with high acid content cause slower digestion by slowing down the emptying of the stomach by causing a reduction in the glycemic index value. Leavening of bread increases acidity, GI for leavened bread is 54, while it is 73 for unleavened bread. Consumption of proteins and fats together with carbohydrates in the diet causes a longer digestion time and insulin secretion and slow digestion of starch are increased by proteins. Fats, on the other hand, cause the food to be present in the stomach longer, prolong the transition time to the intestine, and reduce the glycemic index level. Slow consumption of foods will provide that digestion and absorption will also be slow, which leads to a decrease in GI. It is important to consume protein and fat with large amounts to inhibit the glycemic index from rising. Consumption of 50 grams of fat and 30 grams of protein for 50 grams of carbohydrate can be effective in reduction of the glycemic index. Consumption of foods in the form of puree, paste, canned food, and fruit juice causes the insulin level in the blood to increase more rapidly. As the fruit ripens, the amount of sugar in the fruit increases and the GI value of it increases. GI value of unripe bananas is 31, while GI of ripe bananas is 62 [108]. Blood collection times should be determined in a way for GI determination studies. Blood glucose measurements should be taken until the level of fasting blood glucose is reduced by postprandial blood glucose. In GI determination studies, the time required for the postprandial blood glucose level to reduce to the level of fasting blood glucose in healthy people is 2 hours, whereas in diabetics, this time can be up to 3 hours.

The glycemic response of 50 grams of carbohydrate decreases to the fasting level in 4-5 hours in patients with diabetes and in 1.5 hours in non-diabetic people. Glucose concentrations are different in capillary blood glucose measurement and venous blood glucose measurement. When compared to capillary blood, venous blood has a lower glucose concentration caused by the glucose consumption by skin and skeletal muscle [109]. Healthy individuals and individuals with diabetes have different glycemic responses. Despite the differences in healthy individuals are few, there may be momentary and large deviations in the individuals with diabetes. Patients with type 1 diabetes show higher deviations. Personal differences in the individuals with diabetes cause different glycemic responses [110].

1.11.2. Glycemic Index and Diabetes

There are suggestions that changing the type and amount of carbohydrates consumed in the prevention of type II DM may help maintain β cell function. A critical pathogenic period is thought as worsening of the insulin resistance in time with hyperinsulinemia and β -cell function. Strategies reducing the degree of postprandial glycemia by improving the insulin sensitivity of β -cell function are beneficial [111]. Low amount of glycemic index diets reduce the risk of T2DM in the postprandial period, weight change independently, by lowering demand on β -cells and insulin resistance. Many studies have shown a reduction in blood glucose profile and lower C-peptide levels when comparing low glycemic index diets with high glycemic index diets. It can be seen that low glycemic index diets flourish glycemic control in the patients with diabetes [111]. As to Wolever *et al.*'s study [112], it has been observed that individuals with impaired glucose tolerance have decreased postprandial plasma glucose concentrations at the end of 4 months of a low glycemic load diet. According to the study results of Brand–Miller *et al.* [64]; low glycemic index diets in the patients with diabetes showed 0.4% unit decrease in T1DM patients and 0.2% unit decrease in T2DM patients in the glycosylated hemoglobin levels when compared to high glycemic index diets. The risk of microvascular and neuropathic complications in the patients with T1DM will be efficiently reduced by any reduction in glycemia [113]. A study in the European Insulin Dependent Diabetes Prevention Study (EURODIAB IDDM) investigated the relationship between the glycemic indexes of diets and glycosylated hemoglobin (HbA1C) in 2810 out-patient, European patients with T1DM. As a result of the

current study, it was observed that the patients from southern centers had lower HbA1C levels when compared to the patients coming from northern, eastern, and western centers in terms of the low glycemic index diet consumption. A study showed that the risk of diabetes increased by approximately 60% in women who consumed a high glycemic index diet. Using food-frequency questionnaires in the Nurses' Health Study and The Health Professionals Studies, an inverse relationship between food glycemic index and the risk of diabetes development was shown [114].

1.12. LITERATURE REVIEW

When Rhee *et al.* (2008) tried to gain a standard analytical reference for β -glucan in Chaga (*Inonotus obliquus*) cap mushroom, he compared extraction and analysis methods measured according to β -glucan content. Among different extraction methods (alkaline and enzymatic extraction), two of them were applied to the cell walls of yeast and cereals were used to extract β -glucan. β -glucan content was determined by gravimetric analysis, combined analysis methods, and HPLC (High Pressure Liquid Chromatography) analysis. The contents of β -glucan obtained by both extraction methods were found to be close to each other as seen in all analyzes. A meaning difference was obtained in the β -glucan contents obtained by the same extraction and measured by different analyzes. Thus, it was concluded that the extraction method does not affect the β -glucan content of Chaga mushroom and that the analysis methods should be standardized [115]. Adachi *et al.* (2013) investigated the use of an espresso coffee machine to extract β -glucan from mushrooms. β -glucan was obtained from four mushroom species via extraction with an espresso machine using hot water. Various analytical methods were used to analyze β -glucan, total carbohydrate, protein content, antitumor activity of β -glucan, and antimicrobial activity of tannin. As a result, the highest antitumor activity was found in β -glucan obtained from *Hypsizygus marmoreus* mushroom species. β -glucan obtained by autoclave extraction did not show antitumor activity. The highest β -glucan yield was obtained from *Grifola frondosa* mushroom in both extractions. Via the comparison of the highest yields of β -glucan obtained, it is clear that the yield of β -glucan obtained from the extraction by espresso machine (0.5% by weight) was lower than the utilization of β -glucan gained by autoclave extraction (1.1% by weight) [116].

Harasym *et al.* (2015) aimed to investigate the effect of freezing and then milling via the milling pretreatment applied to oats before the extraction of β -glucan from oats. Oats were frozen at 20°C for 24 hours and then milled. The milled oats were treated with ethanol followed by alkaline extraction method, purification methods, and the β -glucans obtained were dried. Analyses such as viscosity, molecular mass, and β -glucan content were conducted. While the yield of control β -glucan was 54.89%, the yield of β -glucans obtained by freeze milling was 64.03%. β -glucan purity was found to be 54.89% for the control β -glucan and 84.4% for the β -glucans obtained by freeze milling. Molecular mass, viscosity, and water solubility were lower than control β -glucan. It decreased the particle size by 89% and increased the oil excretion by 30%. The rise in surface area with the decrease in particle size, extraction increased proved that oil removal can be possible without the use of enzymes, and it is also effective in reducing the molecular mass [117].

Alzorqi *et al.* (2017) aimed to compare different extraction methods used in the extraction of β -glucan from *G.lucidum* cap mushroom. Thus, three different extraction methods were used: hot water, Soxhlet and ultrasonically assisted extraction. Hot water extraction was carried out at 100°C for different extraction times (1, 2, 3, 4, and 5 hours). Phenol-sulfuric acid, FTIR, SEM (Scanning Electron Microscopy), three different antioxidant assay methods (DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric-Reducing Antioxidant Power) and Folin test and molecular masses were determined to identify the antioxidant activities of the obtained β -glucans. When the extractions were compared with each other, the molecular mass value of β -glucan obtained by ultrasonically assisted extraction (15.995 kDa) and the molecular mass value of β -glucan gained by hot water extraction (15.595 kDa) were found close to each other. The molecular mass value of beta glucan provided by Soxhlet extraction was 12.973 kDa [118].

Tang *et al.* (2017) obtained β -glucan from *S. cerevisiae* cell wall using alkaline-acidic extraction method. The obtained β -glucan was carboxymethylated by treatment with sodium hydroxide, sodium chloride, hydrochloric acid, and ethanol; the obtained β -glucan was phosphatized by treatment with dimethyl sulfoxide (DMSO), phosphoryl chloride (POCl_3) and ethanol; The obtained β -glucan was treated with DMSO, chlorosulfuric acid (HSO_3Cl), pyridine ($\text{C}_5\text{H}_5\text{N}$) and ethanol and binary derivatives were also obtained. The total carbohydrates, molecular masses, antioxidant activities and structures of the derivatives of

β -glucan obtained by six different methods were analyzed for characterization. The lowest molecular mass was found in sulfated β -glucan (2.31×10^4 Da) and the highest molecular mass in carboxymethylated β -glucan (2.97×10^4 Da). The highest total carbohydrate content of 94.17% was obtained in sulfated β -glucan. Sulfated and phosphatized β -glucan showed the best antioxidant activities [119].

Gil-Ramírez et al. (2020) aimed to optimize the conditions of microwave-assisted extraction used in the extraction of β -glucan from *L. edodes*, *A. judea*, *A. aegerita*, *A. caesaria*, *B. edulis*, *C. cibarius*, *C. cornucopioides*, *C. lutescens*, *C. tubaeformis*, *H. marmoreus*, *L. deliciosus*, *M. conica*, *P. eryngii* and *P. pulmonarius* cap mushrooms. The conditions of microwave extraction (temperature and time) were balanced by a three-level and two-factor full factorial design method. Phenol-sulfuric acid for total carbohydrate contents, aniline blue fluorometric analysis for β -glucan contents, GC - MS (Gas Chromatography - Mass Spectrophotometer) and NMR (Nuclear Magnetic Resonance) analysis for monosaccharide compositions were performed. Since the top total carbohydrate and β -glucan content was obtained at 180°C and 30 min extraction conditions, these conditions were determined as the most suitable conditions for the extraction of all mushrooms used [120].

In the study of Nasir et al. (2021), β -glucan was obtained from the fruiting body and mycelium of two different commercial mushrooms (*Pleurotus ostreatus* and *Schizophyllum commune*) via chemical extraction methods on solid waste management (SWM) and then, they were compared. The isolated β -glucan's physical makeup, functional makeup, and characteristics were investigated. The formed conformations of β -glucan and the physical structure were identified and evaluated using Fourier transform infrared (FTIR) and scanning electron microscopy (SEM). Analysis was done on the functional characteristics, swelling power, viscosity, and oil binding ability. The findings revealed that *Schizophyllum commune* micelles had the maximum viscosity (11.85 cP), whereas *Pleurotus ostreatus* micelles had the most ranking swelling power (11.74 g/g) and oil binding capacity (12.09 g oil/g sample) [121].

In the work of Kardono et al. (2013), crude β -glucan from *Tremella fusiform* was isolated, characterized, and tested for glucosidase inhibition to determine its bioactivity. Prior to hot water extraction, contaminants were eliminated using hexane and ethyl acetate in order to

achieve the isolated β -glucan, which had a 33% concentration. The properties of the crude β -glucan were confirmed by FT-IR, ^1H -NMR, and ^{13}C -NMR. According to ^1H -NMR, C-H and CH_2 groups were found between 3.5 and 4.2 ppm. Around 4.8–5.2 ppm, an anomeric proton suggesting glycosidic linkage was seen, and the FT-IR in the 1028 cm^{-1} area verified this. ^{13}C -NMR spectra with chemical shifts of 71.58, 71.87, 72.90, 73.08, 73.47, and 76.53 were the most prevalent, followed by spectra with chemical shifts of 60.41 and 60.95, which suggested CH and CH_2 . At 93.13–102.01 ppm, anomeric carbon that indicates glycosidic carbon was seen. The OH group was detected by FT-IR at $3500\text{--}3000\text{ cm}^{-1}$, followed by the absorption of $\text{C}=\text{O}$ at $1250\text{--}1122\text{ cm}^{-1}$ and C-H at $1500\text{--}1400\text{ cm}^{-1}$ [122].

Although playing a crucial part in the structure of the yeast cell wall, Aimagani et al. (2009) found that the precise composition of the (1 $\rightarrow$ 6)- β -glucan component was little described. A recombinant (1 $\rightarrow$ 3) β -glucanase was used to dissolve the alkali-insoluble portion of the cell wall from a wild-type strain of *Saccharomyces cerevisiae*. The digest was then characterized chromatographically on an anion exchange column. Subsequent analysis revealed that this soluble polymer could only be hydrolyzed by (1 $\rightarrow$ 6) β -glucanase and had a molecular mass of around 38 kDa. Its identity as a (1 $\rightarrow$ 6) gas chromatography-mass spectrometry and NMR (^1H and ^{13}C) investigations established β -glucan polymer with an average branch at every fifth residue and one or two (1 $\rightarrow$ 3) β -linked glucose units in the side chain [123].

According to Lee et al. (2017)'s research, the molecular weight, solubility, and viscosity of β -glucans made from barley pearling by-products were examined. The crude β -glucan extract (45% purity) was subsequently refined around 90% after the first alkaline extraction. With a high molecular weight of under 1106 Dalton, the isolated β -glucans showed a broad molecular weight range [124].

According to the research by Atak (2019), the *in vitro* glycemic index technique was used to assess the GI (Glycemic index) values of several food items and biscuits that are popular in our nation. The research included samples of meals from two separate groups. Red lentils, spaghetti, and rice flour were three distinct food samples included in the first group of the research because they each included varied amounts of fiber, carbohydrate, and starch believed to alter GI. In the second group, three distinct biscuit samples that are known to be

rich in fiber and often eaten as snacks oat-based biscuits, both sugar-free and sweet biscuits were examined to assess the impact of various fiber sources on GI. From marketplaces in Istanbul, samples of red lentils, spaghetti, rice flour, oat-based cookies, sweet biscuits, and sugar-free biscuits were bought. The glycemic indices of white breads, which served as the standard for determining the glycemic index, were 66.2–66.4; nevertheless, the glycemic indexes of food samples ranged from 33.–51.7 and those of biscuit samples from 59.–69.99. Despite being constructed of oats and bran and being light, the sugar-free cookie's GI was discovered to be close to 70 (medium, high) [125].

Mongkontanawat *et al.* research .s (2021) sought to understand the impact of *S. Common* strains, extraction techniques, and β -glucan content and characteristics. The strains *Chanthaburi*, 85-022, 85-023, 85-031, and 85-043 were all used in the study. Hot water extraction and hot alkali extraction were the two distinct β -glucan extraction techniques employed, and they were contrasted with the control [126].

Ahmad *et al.* (2012) shown that β -glucan is a crucial functional component with a number of health advantages. This required component has a variety of known sources. The extract β -glucan from these sources to satisfy the rising demand for nutraceutical goods is thought to be possible. The availability of this functional component makes it possible for entrepreneurs to create new nutraceutical goods. Some goods may provide health advantages beyond just meeting nutritional needs. Scientific studies have shown that β -glucan has a variety of health advantages, from simple stomach issues to sophisticated cancer prevention [127].

By calculating their free sugar component, in vitro starch digestibility, and projected GI, Yaman *et al.* research .s (2019) aimed to assess the nutritional value of several morning cereal variations that are popular in Turkey. The GI varied from 82.6 to 102.8, while the hydrolysis index of breakfast cereals differed from 78.1 to 114.9. Six of the twelve morning cereals had GI values that were greater than those of white bread. While the amount of free sugar, readily convenient glucose, and resistant starch in morning cereals were all strongly associated to changes in GI, there was no connection between quickly and slowly digested starches [128].

Legumes have historically drawn a lot of attention due to their high protein content. Ratnaningsih (2016) asserts that they are favorable due to their low glycemic index (GI), linked to their high amylose and resistant starch levels. The current study's objective was to assess the estimated glycemic index and *in vitro* starch digestibility of five types of cowpeas (*Vigna unguiculata*) grown in Indonesia. Five cowpea varieties were wet milled to remove the starch (KT4, KT5, KT7, KT8, and KTL). Using the Englyst and Goni techniques, the *in vitro* digestibility and eGI of cowpea starches were assessed. Low RDS levels (from 4.09% (KT7) to 7.51% (KT4)) were found in cowpea starches, however high SDS levels (from 19.66% (KT5) to 27.07% (KTL)) and high RS values (from 65.75% (KTL) to 76.15% (KT5)) were found. Cowpea starches have RAG levels between 3.77% (KT7) and 6.79% (KT8) and SAG levels between 18.56% (KT5) and 25.13% (KT8) (KT8) [129].

According to Khanh's (2020) research, the water-soluble polysaccharide β -D-glucans has a wide sort of physiological facilities that make it useful in the food and pharmaceutical sectors. In the current article, it was described how to extract and isolate β -glucan from baker's yeast *Saccharomyces cerevisiae* applying the ionic liquid 1-butyl-3-methyl-imidazolium chloride [BMIM]Cl. The precipitated β -glucans were pure, purified, and free of cell membranes when water and the solution were mixed and recovered by filtering or centrifugation. Once water was added to the combined solution, β -glucan was obtained as white precipitates. The β -glucan product included 78.2% 1,3- β -D-glucan with 98.4% purity, according to the qualitative and quantitative analysis of the ^1D and ^2D NMR spectra and titration techniques [130].

Cross polarization-magic angle spinning (CP-MAS) NMR spectroscopy was used to gain high-resolution solid-state ^{13}C NMR spectra of a range of (1-3)- β -D-glucans in the work by Saita *et al.* (1986). According to the diversity of samples tested, it was discovered that the C-1 and C-3 peak-positions of these glucans are noticeably shifted (up to 8 ppm). These glucans were produced by lyophilization or spray drying, and it's interesting to note that these secondary structures are regular as shown by changes in conformation-dependent ^{13}C chemical shifts. Nevertheless, the capacity of linear (1-3)- β -D-glucans to form gels was dependent only on the presence of one particular conformation and not on any other forms. A branched glucan, on the other hand, that has a shape comparable to a linear glucan without the capacity to produce gels, showed gel formation. Additionally, when solid samples were

created by lyophilization from DMSO solution, it was discovered that all of these linear and branched (1-3)- β -D-glucans had the same structure [131].

1.13. UNIQUE ASPECTS OF THE STUDY

The importance of functional food design is increasing every day. Consumer demand is driving this trend due to increased awareness of the relationship between fiber-rich foods and blood sugar levels, diabetes, obesity, and heart disease. In addition, the successful incorporation of dietary fiber into food products has become significant, with medical studies recommending more fiber in the daily diet to regulate the digestive system. As a result of the increase in health expenditure and the consumer awareness in the field of nutrition and health, and the development of science and technology, the number of studies and the number of products produced in the area of functional food area are improving step by step in time not only in our country but also in the world.

One of the food additives that has emerged as an essential component in the field of functional foods and whose positive effects on health are emphasized is β -glucan. Mushrooms are remarkably nutritious due to containing high-quality proteins, vitamins and minerals. Mushrooms which include low calorie and a little fat and non starchy with a little amount of sugar are very suitable and delightful for obese people. With no starch and very little amount of sugar. Although Turkey is rich in mushrooms, β -glucan is not produced in our country. β -glucan products are imported to be used as food supplements and as food additives in food production processes. β -glucan, which has attracted attention in recent years through its potential contributions to health, is also used as an additive in many foods due to its beneficial effects as well as its gelling and thickening features. Using β -glucan as a food additive in a variety of food industries, including breakfast cereals, prebiotic sausage formulations, sports nutrition products, dairy products like yoghurt, bakery products like cookies, bread, cakes, and ready-to-eat snacks, beverages, salad dressings, and fat substitutes, has a noticeable potential in the near future. These products have some functionalities like a remarkable effect on physical and sensory properties, calorie and cholesterol-reducing effects, and other benefits. In the literature, there are very few studies on the purification and characterization of β -glucan. In the present study, the aim is to purify β -glucan via the alkaline extraction from oyster mushroom and to determine its molecular

weight and viscosity by studying its characterization in detail. This research will also pave the way for the development of efficient techniques to produce of oyster mushroom β -glucan which is an important value-added raw material.

In the last part of the research, the produced β -glucan was incorporated into a bakery product that is a cracker for the first time and the properties of the final product were characterized.

Foods with sufficient β -glucan to make a health claim or provide a overall health benefit to the end user are limited. Therefore, the results of the current research may be an important contribution to the incorporation of β -glucan into foods.



2. MATERIAL AND METHOD

2.1. MATERIALS

Oyster mushrooms were of variety *P. pulmonarius* cultivated in Kütahya and were puched from a local glocery store in Kütahya. Analytical grade chemicals were obtained from Sigma-Aldrich Co. or from Merck.

2.1.1. Moisture Determination

Moisture of fresh mushrooms was detected by using automatic moisture analyzer (Weighed moisture box. *A&D company ltd. N 92; P1011656; Japan*) [132].

2.1.2. Determination of Total Protein

Crude protein was analyzed applying the procedures of American Association of Cereal Chemists (AACC,2000) [133].

2.1.3. Determination of Total Lipid

A slightly modified version of the Folch *et al.* (1957) technique [134] was used to calculate total lipid. A 50 mL combination of chloroform: methanol (2:1 v/v) and five grams of ground up mushroom was added, carefully mixed, and left standing for three days. A table centrifuge was used to filter the solution and centrifugal it at 1000 g. The Pasteur pipette was held to clean the top layer of the methanol, and heating was used to evaporate the chloroform. The crude lipid [135] was all that was left.

2.1.4. Determination of Total Ash

The material was weighed precisely into a crucible at one gram. The crucible was set up on a triangle of clay pipe, burned over a low flame till the whole contents were fully charred, and then cooked in a muffle furnace for almost six hours at 600°C. Following, it was weighed after cooling in a dessicator. After that, total ash was determined using equation [136].

2.1.5. Determination of Total Carbohydrate

With a few minor adjustments, the conventional carbohydrate technique was used to assess the amount of accessible carbohydrates. According to Fox and Robyt (1991) [137], the phenol-sulfuric acid technique was applied to calculate the total amount of carbohydrates.

2.1.6. Determination of Metabolizable Energy Content

Energy is provided through fat, protein or carbohydrates. Total amount of energy was determined by the equations below[138].

Energy(kcal)= 4 X (g protein + g carbohydrate) + 9 X (g lipid);

Energy (kJ)=17 X (g protein + g carbohydrate) +37 X (g lipid)

All calculations were obtained through triplicate (n=3).

2.2. EXTRACTION OF β -GLUCAN FROM MUSHROOM

With a few adjustments, the polysaccharide was isolated from Yap and NG using the technique [139]. Initially, 400 g of fresh oyster mushroom fruiting bodies were cleaned and cut into little pieces. The samples of entire mushrooms were heated with KOH after being cooked in 80% ethanol reflux. Alkali was used by Wei *et al.* to extract β -glucan [140]. Sliced and ground oyster mushrooms using 80% ethanol to induce 6 hours of reflux, mixing the mushroom with KOH (%10, %20, %30, %40), incubating it at progressively higher temperatures (50, 60, 70, 80, 90°C), and letting it sit for progressively longer (30, 60, 90,

120, and 150 min). It is considered that three factors KOH content, extraction temperature, and time are in the interim. With the help of Whatman Nr1 filter paper, the extract was filtered and then concentrated in a rotary evaporator operating at 60 °C at decreased pressure. At the same time, the Sevag reagent (chloroform/butanol, 4:1) removed the proteins in the extract. The concentration was increased by three times by the addition of 95% ethanol. 1500 g centrifuged at 20°C for 10 minutes. The pH of the supernatant was adapted to 10 applying Na_2CO_3 after 20 minutes of centrifugation at 22000 g at 4 °C. A 1:2 mixture of 80% ethanol and supernatant was maintained for 15 minutes. The pellets from a 3500 g centrifugation at 4 °C are dried in a vacuum oven.

2.2.1. Fourier Transform Infrared (FT-IR) Analysis

An FT-IR spectrophotometer was used to identify the purified fractions (FTIR-850, Germany). The polysaccharides were first compressed onto a disk of KBr powder. During the range of 4,000 to 400 cm^{-1} , the spectrum was captured [141]. For instance, the presence of polysaccharides was indicated by peaks in the 950–1,200 cm^{-1} range. The stretching of glycosidic linkages was determined by the peaks at 1,150 and 1,160 cm^{-1} . At 1,376, 1,317, 1,162, 1,100, 1,080, 1,040, and 990 cm^{-1} [141], further peaks and shoulders for β -glucan were discovered.

2.2.2. Nuclear Magnetic Resonance (NMR) Analysis

According to the procedure outlined by Zhu *et al.* (2010) [142], the polysaccharide samples (30 mg) were ultrasonically dissolved in D_2O for 30 minutes before being subjected to ^1H and 125.75 MHz (^{13}C) measurements.

The inability to identify undissolved materials made it difficult to determine the insolubility of particulate β -glucans during NMR analysis. In order to clarify the glucan conformation, a high resolution ^{13}C -NMR of β -glucan particles was carried out in solid state. Powdered β -glucan particles were inserted onto a 3.2 mm Zirconium rotor for NMR examination. A 600 MHz NMR spectrometer was used to record all NMR spectra. Using a Bruker 3.2 mm DVT probe and operating at frequencies of 600.154 MHz for ^1H and 150.154 MHz for ^{13}C . All

studies used a Magic Angle Spinning (MAS) frequency of 10.0 kHz. The precision of the Bruker MAS pneumatic unit's spinning speed control was 2Hz. All tests will employ a 5 s cycle delay. Each cross polarization (CP) experiment required a total acquisition time of 0.017 seconds. The spectral width used to acquire all ^{13}C CP-MAS NMR spectra was 315 ppm. [143].

2.2.3. Liquid-Chromatography-Mass Spectrometry (LC-MS) Analysis

Agilent 1290 RRLC (Rapid Resolution Liquid Chromatography) series equipped with 6550 iFunnel Q-TOF LC/MS system was used to conduct the analysis. From the lowest level of concentration to the greatest level of concentration, β -glucan standards were run. As a blank, pure Milli-Q water was utilized [144].

2.2.4. Antibacterial Activity

One technique for assessing antibacterial activity is the disc diffusion method, which uses 6 mm discs made from Whatmann No:1 paper. During a 24-hour incubation period, 100 L of the test microorganisms were removed and put into petri plates after being adjusted to 1.5×10^8 CFU/mL in accordance with the 0.5 Mc Farland standard. Empty disks were positioned at particular locations on the petri plate and impregnated with 10 L of extract. Positive controls included antibiotic discs made of ofloxacin. To allow for absorption, after being held at +4 °C for 2 hours, the petri dishes were incubated at 37 °C for 24 hours. By measuring the widths of the inhibitory zones on petri dishes, the antibacterial properties of the extracts were discovered [145].

2.2.5. Preparation of Solutions and Rheological Measurement

Several β -glucan concentrations were made by dissolving dried β -glucan powder in distilled water, which was then stirred at 25 °C for 60 minutes on a hot plate magnetic stirrer. The shear rate ramp test was performed employing a cone-and-plate dynamic rheometer with a 40 mm diameter, a 4° cone angle, and a 0.1425 mm gap (Kinexus Dynamic Rheometer,

Malvern, UK). Shear rate ramps with a total ramp length of 2 min and 20 sample points were used to detect shear stress at shear rates ranging from 0.1 s^{-1} to 100 s^{-1} [146].

2.3. PRODUCTION OF CRACKERS

A cracker receipt was prepared by considering the literature; the ingredients of cracker were determined as refined wheat flour, industrial shortening, salt, sugar, and water (Table 2.1) [147].

Table 2.1. The ingredients of cracker

Control	Extracted 5g β – glucan	Extracted 10 g β- glucan	Extracted 15g β - glucan	Extracted 20g β - glucan	Extracted 25g β - glucan	Extracted 30 g β - glucan
250 g rafined wheat flour	245 g rafined wheat flour	240 g rafined wheat flour	235g rafined wheat flour	230g rafined wheat flour	225g rafined wheat flour	220 g rafined wheat flour
50 industrial shorts	50 g industrial shorts	50 g industrial shorts	50 g industrial shorts	50 g industrial shorts	50 g industrial shorts	50 g industrial shorts
5 g salts	5 g salts	5 g salts	5 g salts	5 g salts	5 g salts	5 g salts
112.5 g water	112.5 g water	112.5 g water	112.5 g water	112.5 g water	112.5 g water	112.5 g water

After the cracker ingredients were mixed for 15 minutes in the mixer, the dough flattened with a rolling pin and cut with equal thicknesses in a circular form by using a mold. Crackers placed on the trays cooked at 175°C for 15-20 minutes in an electric furnace. Subsequent to cooling at room temperature, crackers samples put in zipped pouches stored for storage stability study for 3 months in a dark environment and at room temperature.

2.3.1. Chemical Analyses Performed in Crackers

2.3.1.1. Moisture Analysis

Moisture content in the cracker samples determined by utilizing OHAUS MB 45 rapid moisture analyzer (103°C , 1 g sample) [132].

2.3.1.2. Protein Analysis

The amount of protein in the cracker samples performed according to AACC (2000) 46-12 method by using the Kjeldahl method. The results multiplied with the factor of 6.25 and the amount of protein calculated according to the formula [133].

2.3.1.3. Ash Analysis

The amount of ash in cracker samples detected as to AACC Method No: 08-01[136].

2.3.1.4. Determination of Textural Properties of Cracker Samples

The fragility and hardness values of cracker samples produced with the addition of β -glucan were determined with the help of TA. XT Plus Texture Analyzer (England). Pretest velocity (1 mm/s), test velocity (2 mm/s), post-test velocity (10 mm/s), gap (7 mm), and trigger power in analyzes performed with the help of a 3-point bending probe (HDP/3PB) (10 g) is set. Results are expressed as hardness (hardness, g-force) and fracturability (g-force) [148].

2.4. STEPS OF GLYCEMIC RESPONSE DETERMINATION SYSTEM

Determination of glycemic response was carried out after *in vitro* simulated three-stage digestion system called as mouth, stomach, and small intestine [149].

Step 1: Oral digestion (Digestion in mouth)

For *in vitro* oral digestion in the present investigation, a coffee grinder was employed rather of chewing. Two samples of shredded cracker were ground and homogenized separately in the coffee grinder over the course of 0.5 to 1 minutes at room temperature.

Step 2: Stomach digestion

A 100 ml magnetic stirrer was used to combine 0.5 g of guar gum with pepsin enzyme having >250 IU units of enzyme. A little bit of 0.1 N hydrochloric acid (HCl) was added, and a magnetic stirrer was used to agitate it. After then, the volume was finished to 0.1 HCl.

Step 3: Small intestine solution

Intervase (25.43 U/mL), amyloglucosidase (13.4 U/mL), and pancreatin (136 mg/mL). For each sample, 4 mL of deionized water and 680 mg of pancreatin were put to a 50 mL falcon tube. It was vortexed and then centrifuged for 10 minutes at 8000 rpm. The volume was completed to 5 mL after the supernatant was removed, 67 IU of amyloglucosidase enzyme (AMG), and 127.15 U of invertase were added.

Method

Weigh the 250 mL Erlenmeyer with the 1 g homogenized sample. The mixture was then incubated at 37 °C for 30 minutes in a water bath while being shaken with 10 mL of the generated enzyme solution 1 and 5 mL of deionized water added. Before adding 5.0 mL of sodium acetate solution at 0.5 M, the pH was then brought down to 5.2. To create the whole quantity, 5 mL of enzyme solution 2 were combined with 100 mL of deionized water. It was incubated in a shaking water bath at a temperature of 37°C. At 20, 30, 60, 90, 120, and 180 minutes, 0.5 mL of the samples were taken and deposited into a 10 mL glass tube. To denaturize the enzyme, it was kept in a water bath at 100°C for five minutes. The sample was placed in a 15 mL plastic falcon tube with deionized water, and the tube was centrifuged at 8000 rpm for 5 minutes. In a 10 mL glass tube, 3 mL of GOPOD enzyme solution and 0.1 mL of the sample were mixed. They had to wait in a water bath that was heated to 50 °C for 20 minutes before a spectrophotometer measured their absorbance at 510 nm [149].

2.4.1. Hydrolysis Index (HI) Procedure and Expression of HI Value

Using an excel application, the Hydrolyzed Index (HI) value of glucose was determined from the Hydrolyzed Curve for five distinct durations between 0 and 120 minutes as a consequence of *in vitro* digestion of each β -glucan sample. Using the same methods, the HI value of commercial glucose was determined [149].

The HI value of each sample was employed to detect the estimated glycemic index (pGI).

- $AUC = C_{\infty}(t_f - t_0) - (C_{\infty}/k)[1 - \exp[-k(t_f - t_0)]]$

C_{∞} is the ratio of the test sample's area under the hydrolysis curve (AUC) between 0 and 120 minutes to the reference sample's area under the curve (glucose).

The formula used to get the glycemic index value was discovered by Goni and reads as follows: Goni $\{GI = [39.71 + (0.559 \times HI)]\}$.

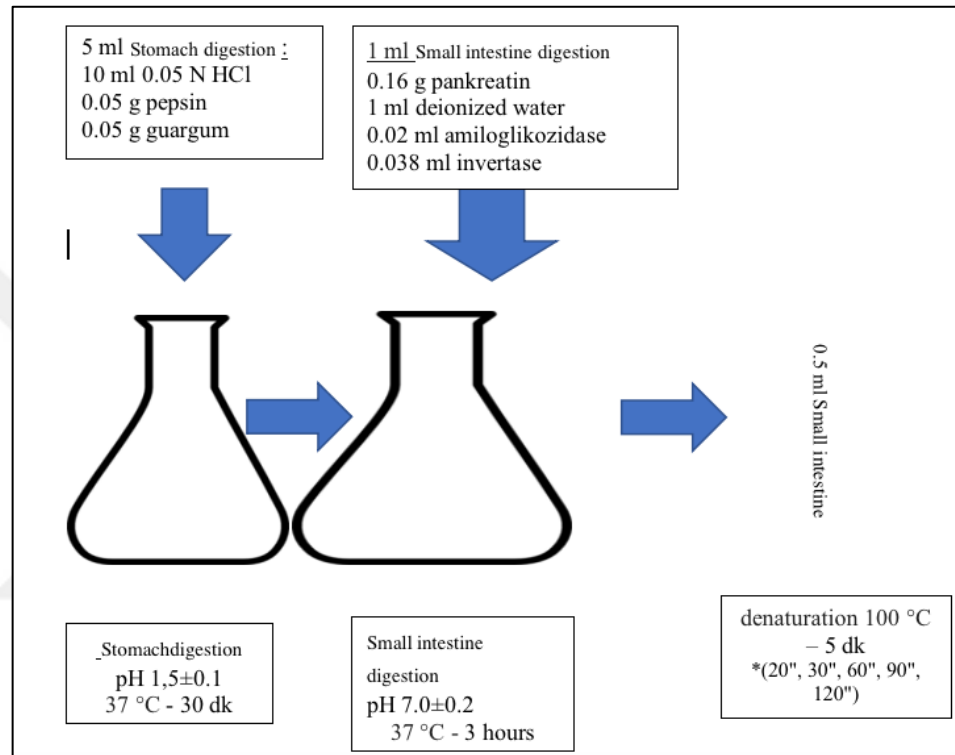


Figure 2.1. *In vitro* glycemic index methode

2.5. SENSORY ANALYSIS

As a result of the literature review, a sensory analysis of β -glucan crackers was conducted using the 1-9 Hedonic scale on a tasting form that was modified for the purpose of the study. Typically, hedonic scales are utilized in consumer tests with untrained panelists. Consequently, it is the most common method for measuring consumers' perceptions of a product. Using hedonic scales, the opinions, preferences, or likes/dislikes of panelists regarding the product can be measured. In this method, after presenting the sample to the panelist in the cabin, the panelist is asked to evaluate the sample by indicating the order of preference or the intensity of the sensory feature on a scale. The evaluated sample is

removed, and a second sample is provided and evaluated. The hedonic scale is the most common method among sensory tests for determining consumer acceptance. The 9-point hedonic scale is the most highly utilized and internationally recognized variant. In this scale, there are nine scales ranging from "I like it extremely" to "I don't like it at all," and consumers are asked to select one of these scales for the offered product. There are two types of 9-point hedonic scales; the first one is a unipolar hedonic scale where scores are given from 1 to 9. Control group and 20% β -glucan crackers were used in the study. An example of this hedonic scale is shown in Table 2.2 [150].

Table 2.2. Verbal hedonic scale example

Name:			Product:		
Panelist No:			Date:		
Instructions: Taste the given samples, then place an X mark on the point in the scale					
	SAMPLE CODE				
SCORE					
Like extremely					
Like very much					
Like moderately					
Like slightly					
Never like nor dislike					
Dislike slightly					
Dislike moderately					
Dislike very much					
Dislike extremely					

2.6. STATISTICAL ANALYSIS

A series of tests were used to determine the statistical differences between the experimental groups. One-way analysis of variance (ANOVA) was used to determine the differences between groups at four different time intervals (30, 60, 90, 120, 150 minutes) and various temperatures (50, 60, 70, 80 degrees). ANOVA is used to test whether the means of independent groups are equal or not.

Pearson correlation analysis was used to evaluate the relationship between time and amount of beta-glucan recovered. This analysis is used to determine the strength and direction of the linear relationship between two variables. The correlation coefficient r determines the

strength and direction of the relationship, while the p-value determines whether this relationship is statistically significant.

All analyses were performed at a significance level where the p-value was less than 0.05, indicating that the results were statistically significant. The statistical software package SPSS was used for data analysis [151].



3. RESULTS AND DISCUSSION

3.1. MOISTURE CONTENT, ASH, CRUDE FAT, CRUDE PROTEIN, CHO, ENERGY (kcal/100g)

Different types of mushrooms have different nutritional qualities in terms of dry matter, moisture, crude protein, ash, organic matter, and energy. As a result of differences in species, maturity stage, substrate, and growth circumstances, mushrooms have different nutritional value and bioactive chemical content. *P. pulmonarius* is very useful as an excellent source of energy, protein, and carbs [151]. The collected data are shown as a brief summary in Table 3.1.

Table 3.1. Results of the oyster mushroom's proximate analysis (*Pleurotus pulmonarius*)

Moisture content(%)	Ash(%)	Crude fat(%)	Crude protein(%)	CHO (%)	Energy (kcal/100g)	Energy (kJ/100)
84.6±0.95	0.89±0.3	0.89±0.3	3.40±0.90	4.75±0.0	40.6±0.00	171.48±0.0

The following values were determined in this study: 84.6% moisture, 40.6% kcal energy, 3.40 % protein, 0.89 % fat, 0.71 % ash, and 4.75 % CHO. According to earlier research, fresh mushrooms contain 90% moisture and 10% dry substance [151, 152, 153, 154, 155]. The study's results are consistent with earlier publications in terms of their values. The species, harvest period, growth, storage circumstances, substrate, and other elements of the growing environment, such as temperature and relative humidity, all affect the moisture percentage of the mushroom [156,157]. According to earlier research, dried mushrooms contain 90% dry matter and 10% moisture, compared to fresh mushrooms' 90% moisture and 10% dry matter [156,157,158,159]. In *Pleurotus* spp., ash concentrations were previously observed to range from 4.4 to 13.7% [89, 159, 160, 161, 162, 163]. The ash content of *Pleurotus pulmonarius* was found to be comparable to the ash content in these investigations. Since amino acids highly exist in them and have a higher nutritional value

than vegetables, proteins made from mushrooms have recently caught the interest of individuals in the food business and the scientific community [164].

Table 3.2. Results of the oyster mushroom's proximate analysis correlations

		Moisture content (%)	Ash (%)	Crude fat(%)	Crude protein (%)	CHO (%)	Energy (kcal/1)	Energy (kJ/100)
Moisture content(%)	r	1	1,000**	1,000**	1,000**	1,000**	-1,000**	1,000**
	p		0	0	0	0	0	0
	N	3	3	3	3	3	3	3
Ash(%)	r	1,000**	1	1,000**	1,000**	1,000**	-1,000**	1,000**
	p	0		0	0	0	0	0
	N	3	3	3	3	3	3	3
Crude fat(%)	r	1,000**	1,000**	1	1,000**	1,000**	-1,000**	1,000**
	p	0	0		0	0	0	0
	N	3	3	3	3	3	3	3
Crude protein(%)	r	1,000**	1,000**	1,000**	1	1,000**	-1,000**	1,000**
	p	0	0	0		0	0	0
	N	3	3	3	3	3	3	3
CHO(%)	r	1,000**	1,000**	1,000**	1,000**	1	-1,000**	1,000**
	p	0	0	0	0		0	0
	N	3	3	3	3	3	3	3
Energy (kcal/100)	r	-1,000**	-1,000**	-1,000**	-1,000**	-1,000**	1	-1,000**
	p	0	0	0	0	0		0
	N	3	3	3	3	3	3	3
Energy (kJ/100)	r	1,000**	1,000**	1,000**	1,000**	1,000**	-1,000**	1
	p	0	0	0	0	0	0	
	N	3	3	3	3	3	3	3

**. Correlation is significant at the 0.01 level (2-tailed).

Note. Since the number of trials is very low, the relationship between the variables cannot be tested statistically.

Consequently, using edible mushrooms to create protein-rich food items might increase their consumption while providing a tasty substitute for animal protein [165]. *P. pulmonarius* provided the research with the value for crude protein (3.4%) (Table 3.2). In *Pleurotus* species, protein content has been recorded to range from 1.4 to 44.3% [153,158,161]. The genus of the mushroom, the time of post-harvest analysis, and the stage of development are all factors that affect the protein content of mushrooms [166, 167]. Additional elements include the variations in the growth environment's physical and chemical composition, the kind and nutritional content of the substrate used for culture, and the species of the mushroom. Mushrooms typically contain relatively little fat; the unsaturated fatty acids dominate the fat content. According to Table 3.1, *P. pulmonarius* has a crude fat content of

0.89%. In *Pleurotus* spp., fat levels have been observed to range from 0.85 to 3.16 % [168, 169, 170]. As a highly regarded excellent source of carbohydrates, edible mushrooms typically contain 3.7% to 76.2% of their dry weight [151,153, 158, 159, 162, 163, 169, 171]. *P. pulmonarius* was estimated to have an energy value of 40.6 kcal. When the acquired data were compared to those from other investigations [161, 163, 172], it was discovered that *P. pulmonarius* had the same energy value.

3.2. EXTRACTION OF β -GLUCAN FROM MUSHROOM

With more N- and O-type carbohydrate-peptide connections were broken as KOH concentration rose, which led to the release of more polysaccharide. Nevertheless, the structure of the polysaccharide was harmed when the KOH concentration rose over 30%. Protein denaturation and a decrease in extraction rate were both caused by high temperature. Another element influencing polysaccharide extraction is extraction time. Polysaccharide degradation was brought on by prolonged extraction time. Hence, 90 minutes was the ideal extraction time. Table 3.3 lists the reported findings for various extraction settings.

Based on the rate of polysaccharide extraction, alkaline extraction was chosen as the optimum technique. The extraction procedure was carried out at increasing KOH concentrations (0, 10, 20, 30 and 40%) for increasing periods at increasing incubation temperatures (50, 60, 70, 80, and 90°C) and at increasing duration of time (30, 60, 90, 120 and 150 min). In the meanwhile, three variables, we considered KOH concentration, extraction temperature, and extraction time. Three times each element was checked. The extract was filtered using Whatman Nr1 filter paper, and the filtrate was then concentrated in a rotary evaporator operating at 60 °C at reduced pressure. Sevag reagent was used to extract the proteins from the extract, and after Sevag reagent was removed, the concentrate was mixed with three times its volume of 95% ethanol before being left to stand at 4°C overnight to precipitate polysaccharides. To extract crude polysaccharides by lyophilization, the precipitate was collected after being centrifuged at 3,000 rpm for 10 min. It was then dialyzed with flowing water for 48 h.

Table 3.3. Extraction yield%

%KOH	Temperature(oC)	Time (min)				
		30	60	90	120	150
10%	50	0.025	0.0375	0.05	0.05	0.125
	60	0.125	0.05	0.0625	0.055	0.175
	70	0.137	0.06	0.075	0.0875	0.225
	80	0.175	0.077	0.0875	0.0925	0.275
20%	50	0.055	0.05	0.075	0.1	0.125
	60	0.075	0.10	0.11	0.125	0.275
	70	0.075	0.11	0.125	0.137	0.3.
	80	0.125	0.15	0.175	0.187	0.325
30%	50	1.25	1.75	2.55	2.25	2.
	60	1.50	2.00	3.90	2.5.	2.5
	70	1.75	2.00	4.20	2.25	2.53
	80	2.25	2.37	5.50	3.12	2.52
40%	50	1.25	1.50	2.37	2.12	1.75
	60	1.40	1.75	2.45	2.25	2.00
	70	1.50	2.25	2.55	2.25	2.00
	80	2.00	2.25	2.50	2.00	1.75

In conclusion, 30% (w/v) KOH concentration, 80°C, and a 90 min extraction time were the ideal extraction parameters. Hence, 90 minutes was the ideal extraction period. The isolated solution's protein was eliminated using the Sevag reagent [173]. Only by isolating purified β -glucan from the plant matrix using various extraction methods is it possible to do so. Several extraction stage pHs, such as acidic, neutral, and alkaline, may be used in wet extraction methods. Alkaline water processes provide the best yield of extracted β -glucan despite the fact that the process has distinct properties [174, 175, 176]. At neutral pH and notably alkaline pH, a simultaneous rise in protein solubility was seen [177,178].

Table 3.4. Measurements in time and four different concentrations

		N	X	ss	F	p
Time (30)	10%	4	0.1155	0.06399	41.925	0.000
	20%	4	0.0825	0.02986		
	30%	4	1.6875	0.42696		
	40%	4	1.5375	0.32500		
	Total	16	0.8558	0.82011		
Time (60)	10%	4	0.0561	0.01669	93.196	0.000
	20%	4	0.1025	0.04113		
	30%	4	2.0300	0.25547		
	40%	4	1.9375	0.37500		
	Total	16	1.0315	1.00507		
Time (90)	10%	4	0.0688	0.01614	40.552	0.000
	20%	4	0.1213	0.04151		
	30%	4	4.0375	1.21063		
	40%	4	2.4675	0.07676		
	Total	16	1.6738	1.81172		
Time (120)	10%	4	0.0713	0.02184	146.636	0.000
	20%	4	0.1373	0.03657		
	30%	4	2.5300	0.41061		
	40%	4	2.1550	0.12014		
	Total	16	1.2234	1.17993		
Time (150)	10%	4	0.2000	0.06455	200.327	0.000
	20%	4	0.2563	0.08985		
	30%	4	2.3875	0.25863		
	40%	4	1.8750	0.14434		
	Total	16	1.1797	1.01058		
Mean	%10	4	0.1023	0.03571	96.218	0.000
	%20	4	0.1400	0.04591		
	%30	4	2.5345	0.48801		
	%40	4	1.9945	0.14570		
	Total	16	1.1928	1.14747		

In their 2014 study, Vizhi and Many reported yields of 5.4%, recovery of 83.8%, and compositions of protein (7.02%), soluble dietary fiber (87.52%), insoluble dietary fiber (6.01%), fat (0.79%), and ash (1.2%) (179). A research indicated that the Thrace Region's edible mushroom species have a levels of β -glucan *A. bisporus* at a $14.57 \pm 0.80\%$, $13.93 \pm 0.78\%$ *B. edulis*, $12.89 \pm 0.84\%$ *C. cibarius*, $12.84 \pm 0.11\%$ *H. repandum*, and $11.26 \pm 0.69\%$ *C. curnicopioides* [180]. Moreover, Manzi *et al.* (2000) discovered that 0.22% to 0.53% of *Pleurotus* species contained β -glucan [181]. When market-cultivated mushroom species such Morchella, Boletus, Shitake, and Maitake were tested, it was found that their β -glucan concentration ranged between 7.9% and 48.3% [182]. The values were found to be

39.78, 39.15, 47.11, and 56.44%, respectively, in a research in which the contents of β -glucan in short and long stemmed *Pleurotus abalonus*, *Pleurotus eous*, and *Auricularia auricula* species were examined [182].

Table 3.4 presents measurements taken for time intervals (30, 60, 90, 120, and 150 min) and four different concentrations (10%, 20%, 30%, and 40%). In the table, N represents the sample size, X denotes the mean measurement value, ss represents the standard deviation, F represents the ANOVA test statistic, and p represents the p-value.

There are several important points that can be inferred from the table:

For each time interval, there are significant differences between the different percentage groups. This is confirmed by the F value and the p-value for each time interval. In every case, the p-value is much lower than 0.05, indicating that the differences are statistically significant. As the time interval increases, there is a general increase in the mean measurement values. This indicates that the system or process is changing over time. Within each percentage group, the mean measurement values generally increase, but this increase is not always equal. For example, the mean values are generally lower in the 10% group, but higher in the 30% and 40% groups. This may indicate that the percentage groups affect the process in different ways. The standard deviation values generally vary across the percentage groups and time intervals, indicating that the distribution of the measurement values changes depending on these factors.

The ANOVA test indicates a significant difference among all time intervals and percentage groups. The F value is generally large, and the p-value is much smaller than 0.05, indicating that these differences are statistically significant.

Table 3.5. Measurements taken over time and temperature ranges

		N	X	ss	F	p
Time (30)	50 °C	4	0.6450	0.69870	0.218	0.882
	60 °C	4	0.7750	0.78076		
	70 °C	4	0.8655	0.88328		
	80 °C	4	1.1375	1.14501		
	Total	16	0.8558	0.82011		
Time (60)	50 °C	4	0.8344	0.91864	0.086	0.966
	60 °C	4	0.9750	1.04443		
	70 °C	4	1.1050	1.18238		
	80 °C	4	1.2118	1.6945		
	Total	16	1.0315	1.00507		
Time (90)	50 °C	4	1.2613	1.38618	0.110	0.953
	60 °C	4	1.6306	1.87907		
	70 °C	4	1.7375	2.00733		
	80 °C	4	2.0656	2.54762		
	Total	16	1.6738	1.81172		
Time (120)	50 °C	4	1.1300	1.21954	0.020	0.996
	60 °C	4	1.2325	1.32350		
	70 °C	4	1.1811	1.23440		
	80 °C	4	1.3499	1.47075		
	Total	16	1.2234	1.17993		
Time (150)	50 °C	4	1.0000	1.01550	0.047	0.986
	60 °C	4	1.2375	1.18752		
	70 °C	4	1.2638	1.17662		
	80 °C	4	1.2175	1.10528		
	Total	16	1.1797	1.01058		
Mean	50 °C	4	0.9741	1.04699	0.075	0.972
	60 °C	4	1.1701	1.23586		
	70 °C	4	1.2306	1.27971		
	80 °C	4	1.3965	1.48344		
	Total	16	1.1928	1.14747		

The 3.5. table includes measurements taken across different time intervals (30, 60, 90, 120, and 150 min) and temperature degrees (50, 60, 70, and 80 °C). There are several important points that can be inferred from the table: For each time interval, there is no significant difference among the different temperature degrees. This is confirmed by the F value and the p-value for each time interval. In every case, the p-value is greater than 0.05, indicating that the differences are not statistically significant. The mean measurement values generally vary across temperature degrees and time intervals. This indicates that the process or system responds differently to different temperature degrees and time intervals.

For each temperature degree, the mean measurement values generally increase. However, this increase is not always equal. For example, the mean values are generally lower in the 50°C group, but higher in the 80°C group. This may indicate that the temperature degrees affect the process in different ways.

The standard deviation values generally vary across temperature degrees and time intervals. This indicates that the distribution of the measurement values changes depending on these factors.

The ANOVA test indicates that there is no significant difference among the different temperature degrees and time intervals. The F value is generally small, and the p-value is greater than 0.05, indicating that these differences are not statistically significant.

The information provided in this table helps in understanding the effect of each temperature degree and time interval on the process. However, the overall conclusion is that the process or system is not sensitive to temperature degrees, and there is no statistically significant difference among these factors. However, these results may not apply to a particular situation, as the measurements represent general trends and not a specific process or system.

Table 3.6. Measurements taken at different time intervals

	N	Minimum	Maximum	Mean	Std. Deviation	F	p
Time (30 min)	16	0.03	2.25	0.8558	0.82011	7.486	0.009
Time (60 min)	16	0.04	2.37	1.0315	1.00507		
Time (90 min)	16	0.05	5.50	1.6738	1.81172		
Time (120 min)	16	0.05	3.12	1.2234	1.17993		
Time (150 min)	16	0.13	2.53	1.1797	1.01058		

This table includes measurements taken across different time intervals (30, 60, 90, 120, and 150 time units). N represents the sample size, Minimum and Maximum represent the lowest and highest measurement values, Mean represents the average measurement value, Std. Deviation represents the standard deviation, F represents the ANOVA test statistic, and p represents the p-value. There are several important points that can be inferred from the table: Variations can be observed in the measurements for each time interval, which can be understood from the minimum and maximum values. These values generally increase as the time interval increases. The mean measurement values generally increase as the time interval

increases. This indicates that the process or system changes over time. The standard deviation values also generally increase as the time interval increases. This indicates that the distribution of the measurement values spreads out more over time. The ANOVA test indicates a significant difference among the different time intervals. With a large F value and a p-value smaller than 0.05, it can be stated that these differences are statistically significant.

The alkaline extraction method yielded the highest yield and purity, ensuring maximum removal of impurities. Additionally, this method resulted in the highest amount of soluble β -Glucan. In a conducted study, it was determined that a NaOH concentration above 0.5 M, extraction temperature of 135°C, and extraction time of 45 minutes were the ideal extraction parameters [183]. These ideal extraction parameters were further confirmed by FTIR analysis (Figure 3.1).

3.2.1. Fourier Transforms-Infrared Spectroscopy (FT-IR Analysis)

The functional groups like OH-, C-H, and C-O of the crude β -glucan were identified using Fourier Transforms-Infrared spectroscopy (FT-IR). The absence of water-soluble glucan absorption bands in the infrared spectrum, which are characteristic of β -(1--3)/(1--6)-glucans. In Figure 3.1, the FIR spectrum is shown.

Table 3.7. The content of β -glucan

Bond area	Functional group
3301.906 cm^{-1}	OH
2921.754 cm^{-1}	CH
1639.486 cm^{-1}	OH
1368.359 cm^{-1}	CH ₂ , CH, OH
1015.685 cm^{-1}	CO, CC, COH(β -anomers of glucans)

The linkages in the area of 3301.906 cm^{-1} , 2921.754 cm^{-1} , 1639.486 cm^{-1} , and 1368.359 cm^{-1} in β -glucan were typical polysaccharide absorptions (Table 3.7). Stretching of the OH-bond was ascribed to the band at 3301.901 cm^{-1} . The bending modes of C-H and OH gave rise to the following bands at 2921.754 and 1639.486 cm^{-1} . Many bands below 1015.685 cm^{-1}

¹ belong to ring and skeleton modes, whereas intensely overlapped bands in the range of 1015.685 cm^{-1} relate to CO, CC stretching, and COH bending modes. The presence of a band at 890 cm^{-1} is a distinct characteristics of polysaccharides with a β -configuration, specifically indicating the (C1-H) deformation mode. Additionally, there are absorption bands at 2920, 1370, 1250, and 1200 cm^{-1} that differentiate 1---3 linkages. The absorption at 1160 cm^{-1} corresponds to (C-C) and (C-O-C) stretching vibration, while two partially overlapping bands at 1078 cm^{-1} and 1048 cm^{-1} are associated with ring stretching and (C-OH) side group stretching, respectively [184].

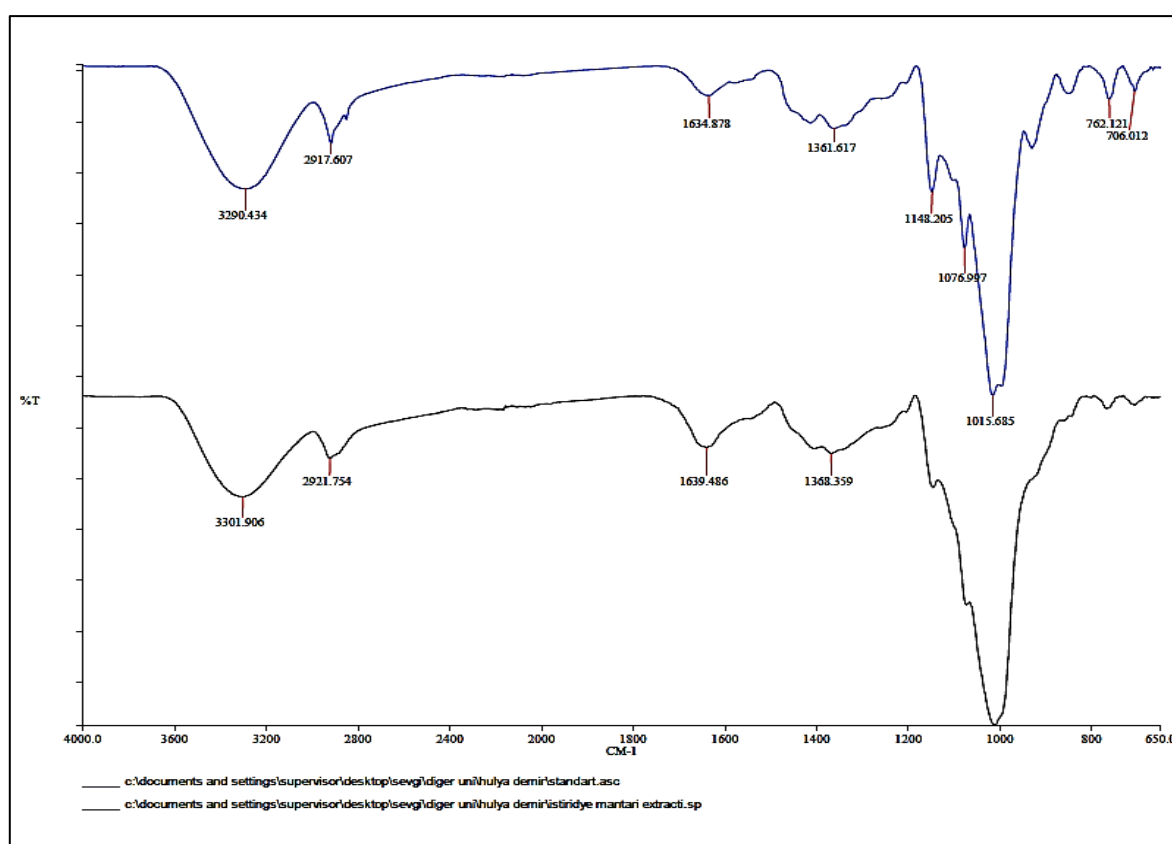


Figure 3.1. FTIR analysis of sample and standard

Free hydroxyl groups absorb in the region of 3301.906 cm^{-1} . The hydroxyl groups involved in hydrogen bond formation caused the maximum position of the band to shift to lower frequencies, increasing its intensity and broadening the band, while at the same time, also caused symmetry distortion.

3.2.2. Nuclear Magnetic Resonance Spectroscopy (NMR)

In order to reveal the conformational properties of crystalline as well as non-crystalline samples, high-resolution solid-state ^{13}C NMR spectroscopy has recently proven to be very useful for various types of molecular systems [185].

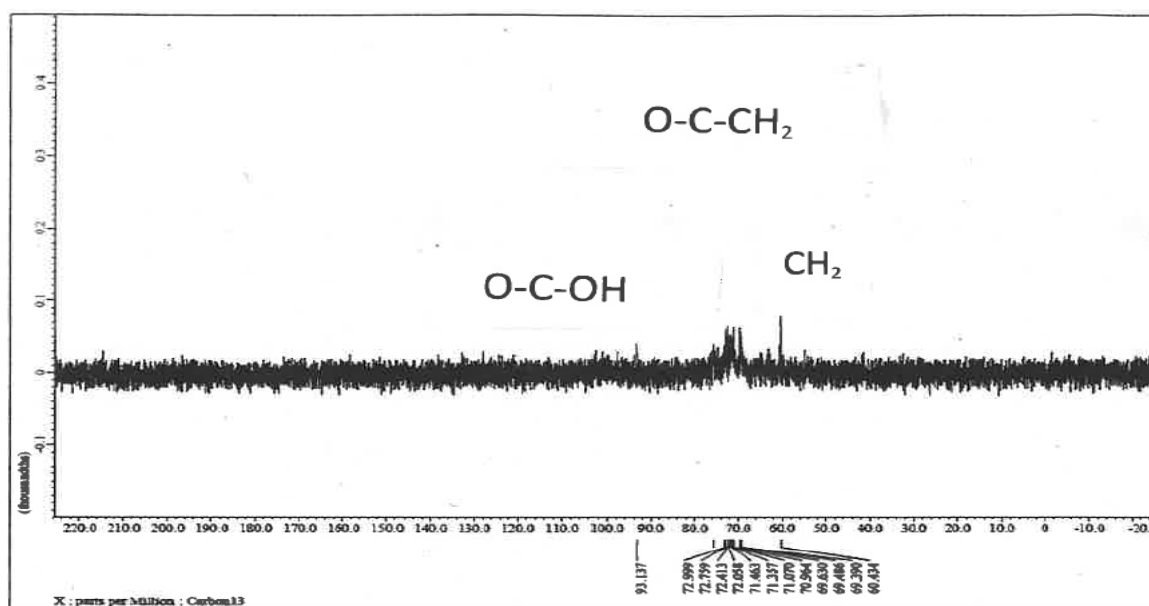


Figure 3.2. ^{13}C NMR spectrum β -glucan sample (a)

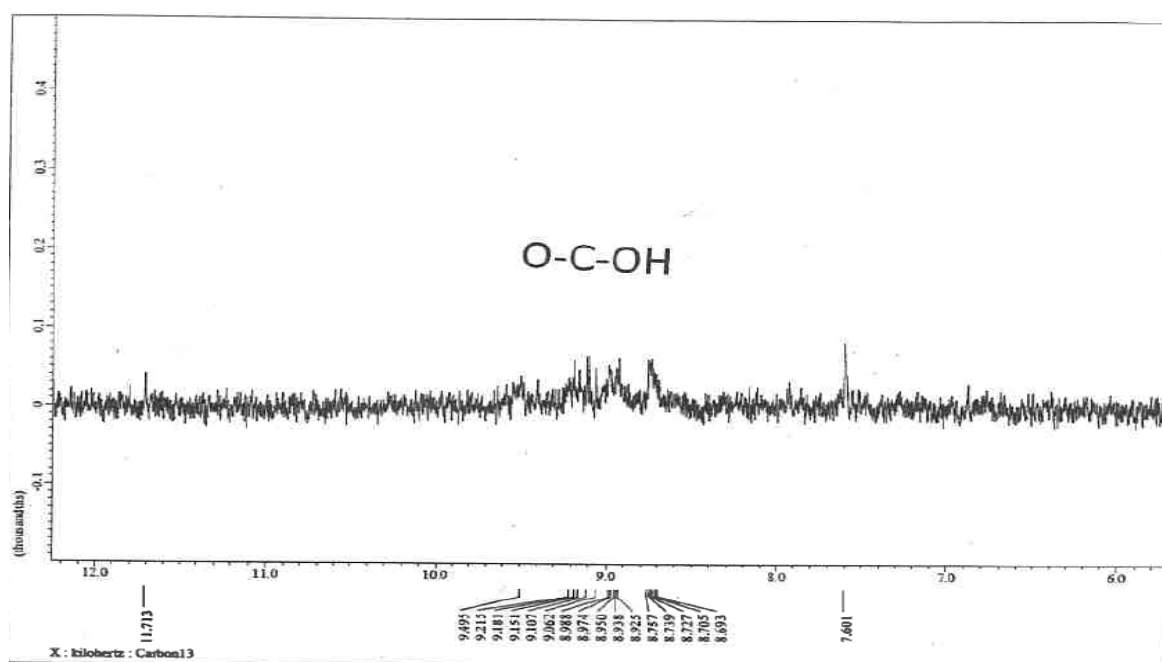


Figure 3.3. ^{13}C NMR spectrum β -glucan sample(b)

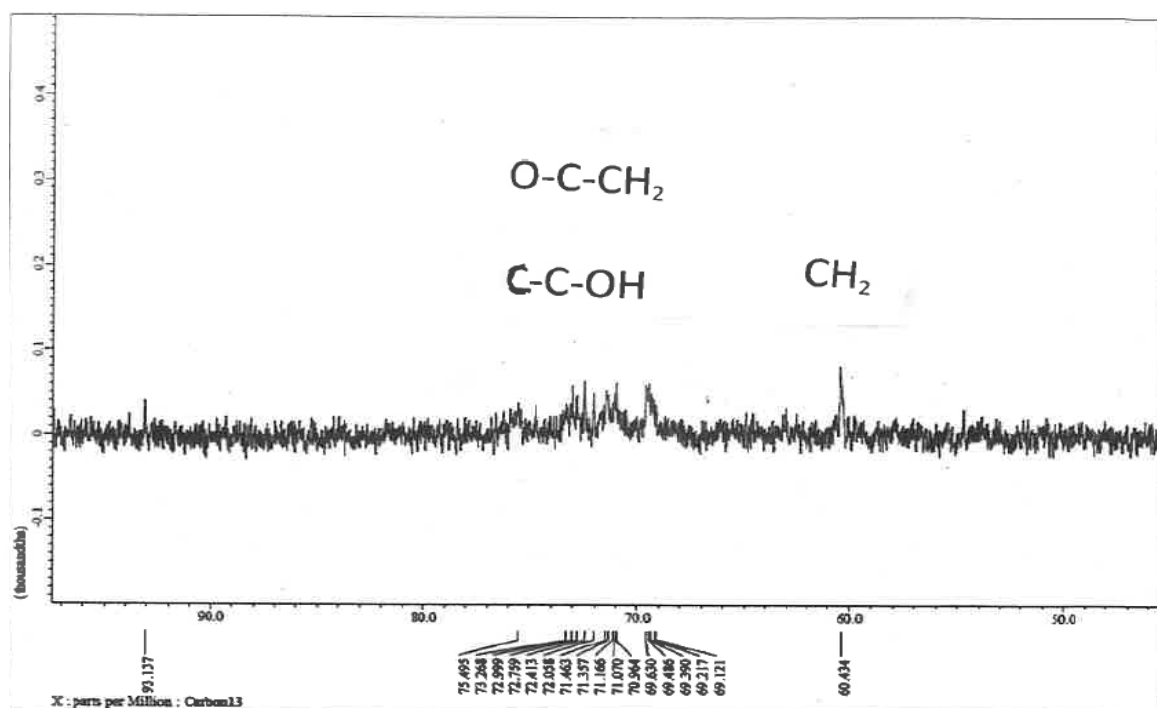


Figure 3.4. ^{13}C NMR spectrum β -glucan sample (c)

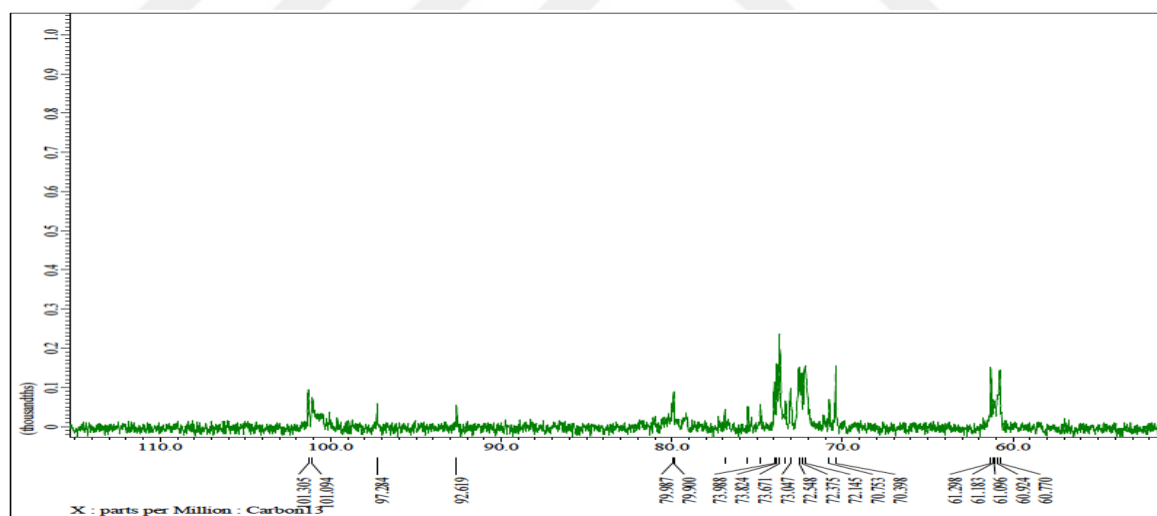


Figure 3.5. ^{13}C NMR spectrum β -glucan standard (a)

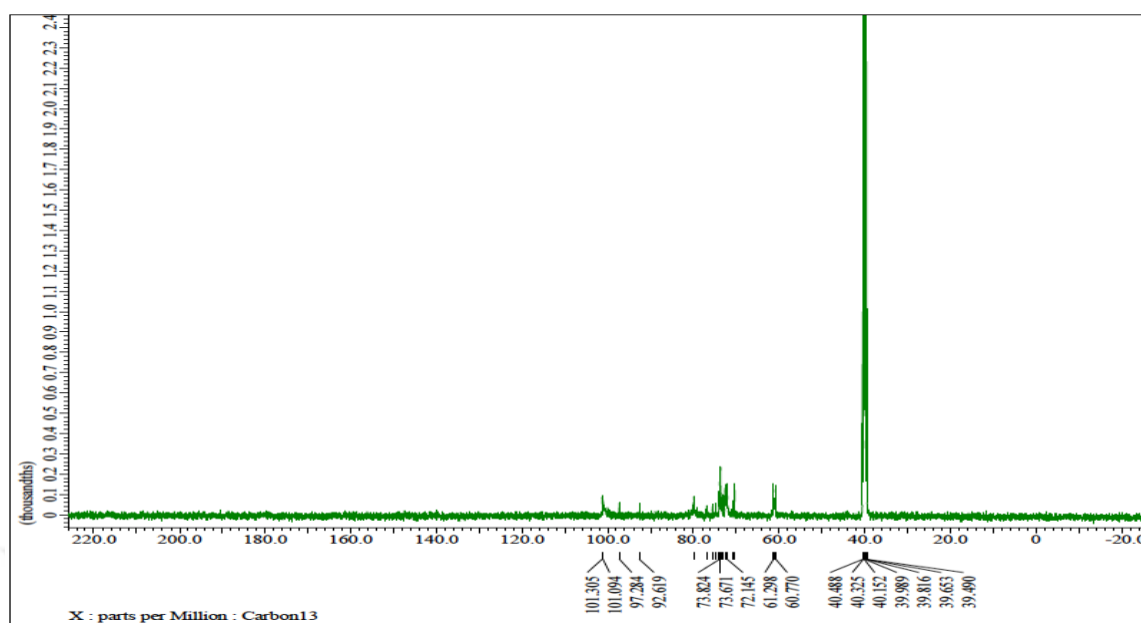


Figure 3.6. ^{13}C NMR spectrum β -glucan standard (b)

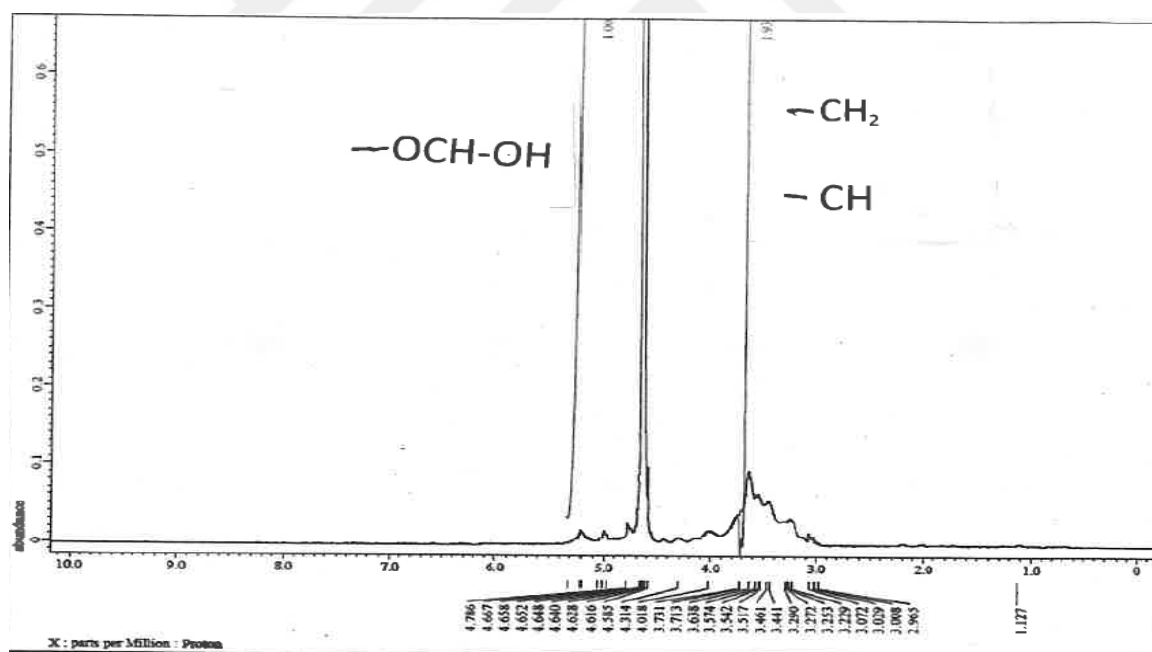


Figure 3.7. ^1H -NMR spectrum β -glucan sample (a)

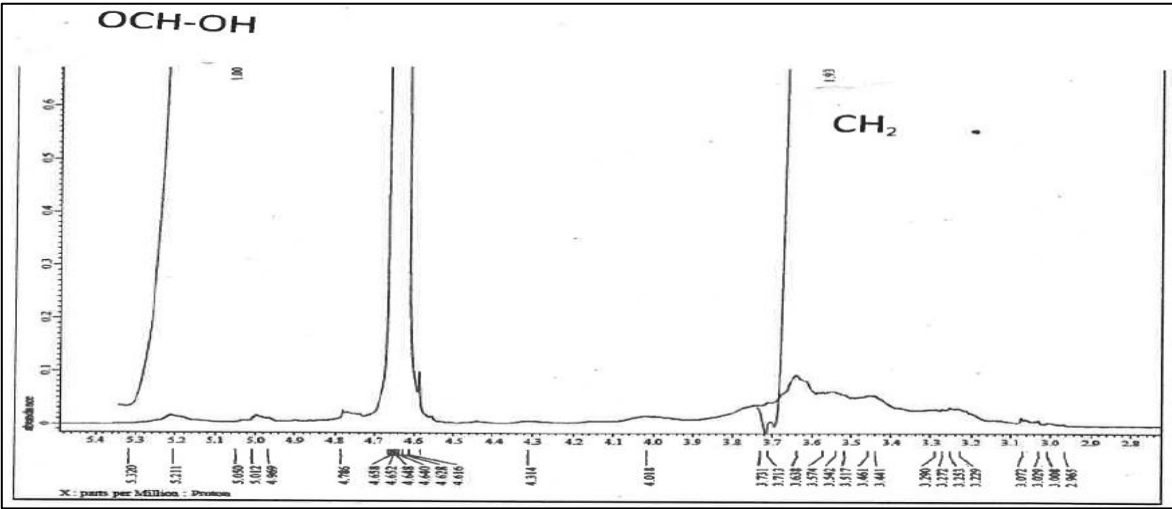


Figure 3.8. ¹H-NMR spectrum β-glucan sample (b)

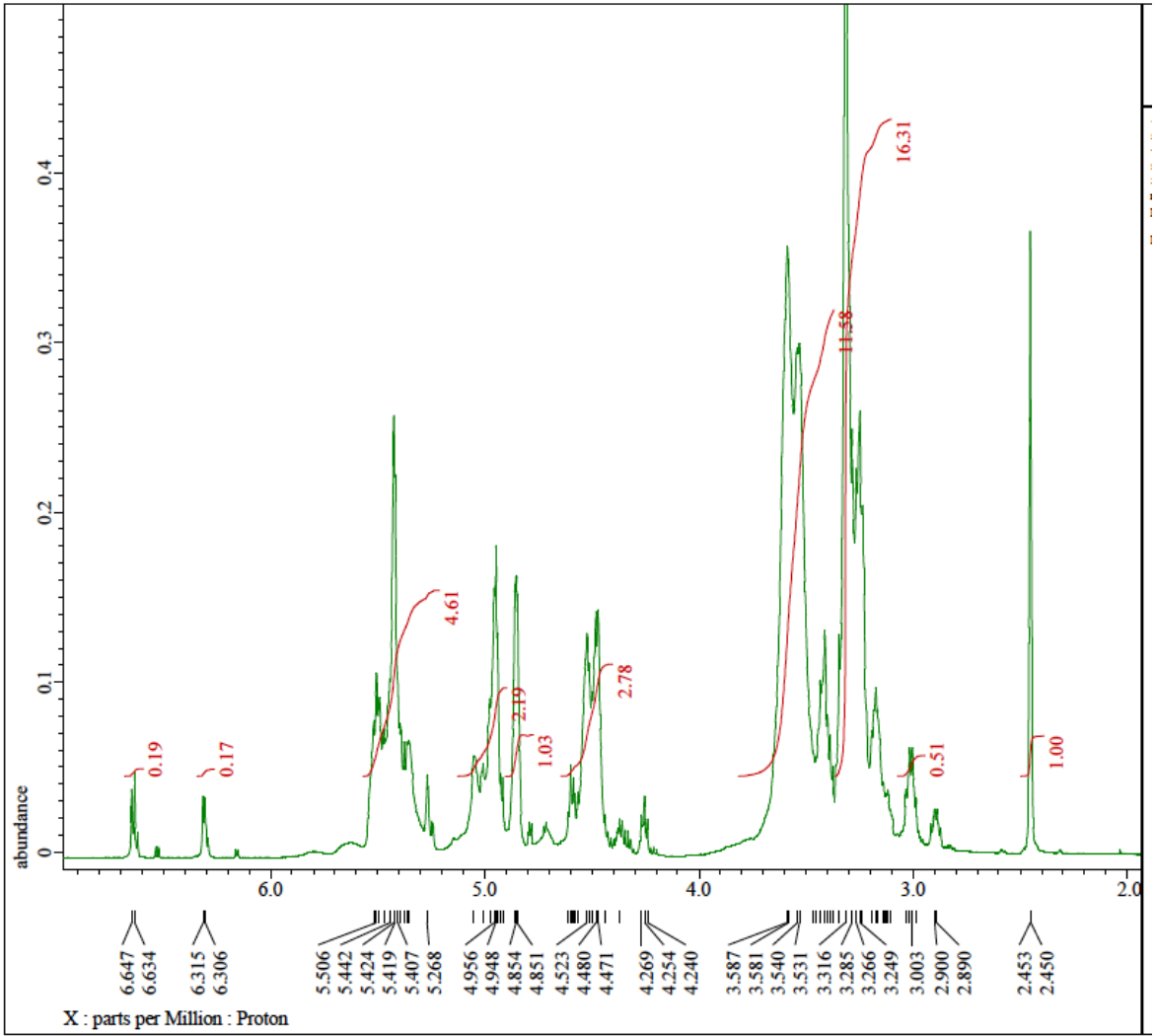


Figure 3.9. ¹H NMR spectrum β-glucan standard (a)

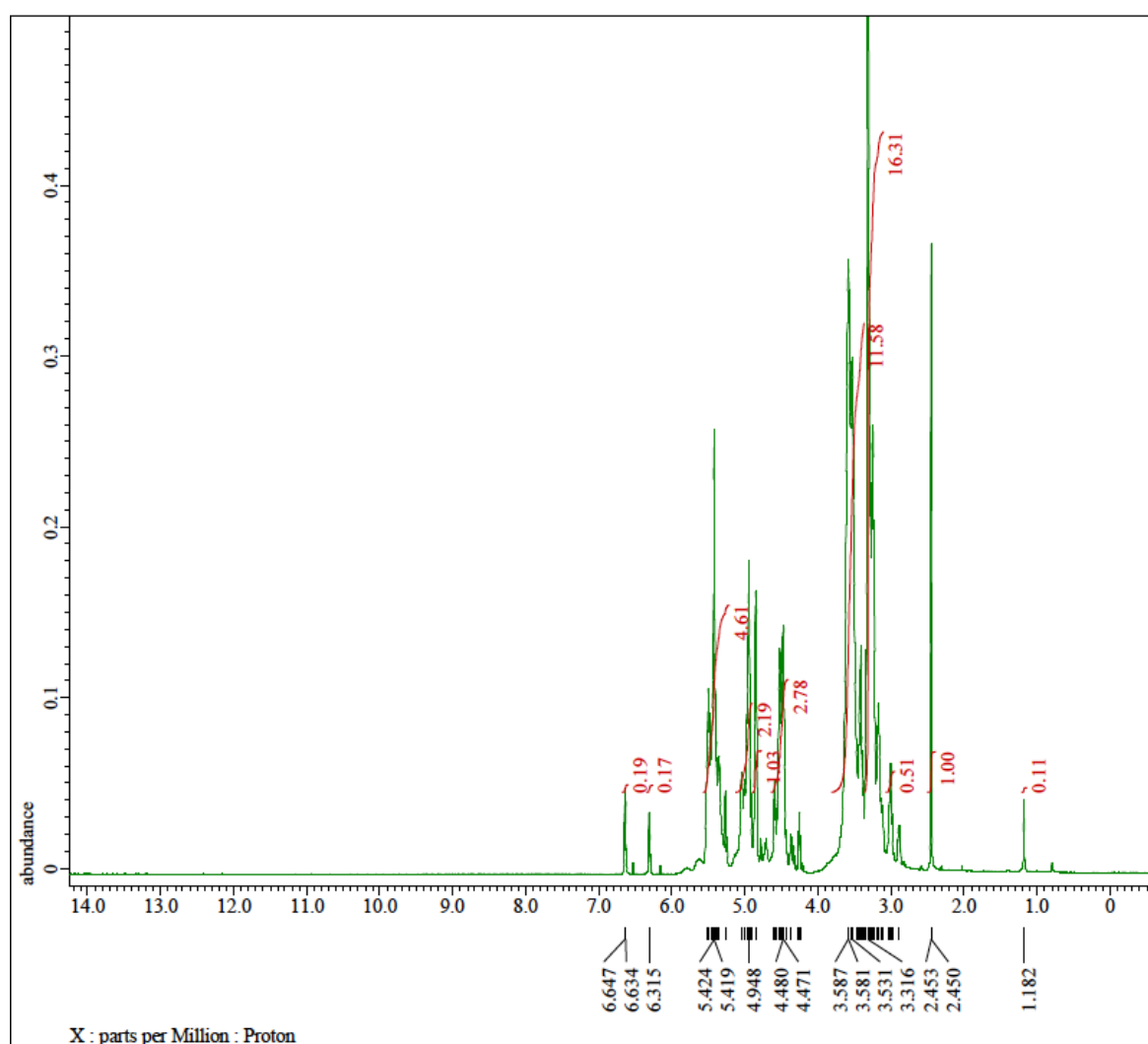


Figure 3.10. ^1H - NMR spectrum β -glucan standard (b)

Analyses were taken under room temperature by using a standard 5 mm NMR tube at the JEOL ECZ 500 R NMR device. The sample was dissolved in D_2O . The number of screening was adjusted as 32 and the clarification time as 5 seconds.

According to the ^1H NMR spectrum of the sample, it was observed that there were impurities in the sample. However, a trace of humidity was also present in the sample. Besides all of them, the solubility of the sample in D_2O was quite low. Due to the fact that the analyses were taken in the D_2O solvent, the protons of the OH groups in the structure exchanged protons with the Deuterium atoms in D_2O , thus OH peaks could not be observed in the ^1H NMR spectrum. In addition, it was thought that the protons of the CH_2 group structure reached at 3.638 ppm. The protons of the CH groups within the group came quite close

between 3.1 ppm and 4.0 ppm. CH groups were also close to each other due to the fact that they were remarkably similar in terms of their chemical environment. CH peak in O-CH-OH group was thought to have come at 5.32 ppm. Other incoming peaks came from the impurities in the environment.

According to the ^{13}C NMR spectrum of the sample, the peak of the C atom in O-C-OH group was observed at 93.137 ppm (11.713 kHz) and the peak of the C atom in O-C-CH₂ group was observed at 75.495 ppm (9.495 kHz). The peaks of the C atoms in three different C-C-OH groups in the structure were observed at 72.999 ppm (9.181 kHz), 72.413 ppm (9.107kHz), and 72.058 ppm (9.062 kHz), respectively. The peak of the C atom of CH₂ group was detected at 60.434 ppm (7.601 kHz). All of the monosaccharide residues in oyster mushrooms have anomeric configurations, as shown by resonances of 93.137 ppm that are assigned to anomeric carbon atoms. There are no carboxyl, acetyl, or amido groups in β -glucan since no chemical change was seen over 170 ppm.

The water-soluble glucan's ^1H NMR spectrum is shown in Figure 3.8. Due to the existence of the macromolecule's -anomeric form, a strong peak (4.5 ppm) in the spectrum is seen. These results agree with those from FTIR spectroscopy.

Proton magnetic resonance chemical shifts between 3.00 and 4.50 ppm show that this polymer is a carbohydrate-based molecule. The existence of ^1H , 1-3- β -linked glucopyranose and 4.36 ppm ^1H , 1-6- β -linked glucopyranose is indicated by proton signals in the spectra of anomeric carbon atoms at 4.47 ppm and 4.36 ppm ^1H , respectively (Figure 3.8). Its structure is the same as that of other D-(1 $\rightarrow$ 3)/(1 $\rightarrow$ 6)- β -glucans. Its primary chain is made up of linked (1 $\rightarrow$ 3)-glycoside linkages made from the residues of D-glucopyranose.

Table 3.8. Chemical shifts of signals in ^{13}C NMR spectra of glucan

Carbohydrate residues	Chemical shifts, ppm					
	C1	C2	C3	C4	C5	C6
3,6- β -Glc-(1-3)	93.137	7.413	72.159	69.486	73	69.63

The data obtained were similar to those described in the literature [185]. Based on the results obtained, it was concluded that the water-soluble glucan macromolecule has a branched structure, the basis of which consists of D-glucopyranose residues linked by β -(1 $\rightarrow$ 3)-

glucoside bonds. Lateral branches, built from β -glucopyranose monomer units, were attached to the O-6 positions of monosaccharide residues.

In the current study, the proton magnetic resonance chemical shifts in the range of 3.00-5.35 ppm suggest that the polymer being investigated possesses a carbohydrate structure. The presence of proton signals in the spectrum of anomeric carbon atoms at 4,413 ppm indicates the presence of ^1H , β -1 $\rightarrow$ 3- linked glucopyranose and at 4,018 ppm ^1H β -1 $\rightarrow$ 6-linked glucopyranose. Results are consistent with the literature [186, 187,188].

3.2.3. Liquid-Chromatography Mass (LC-MS) Analysis

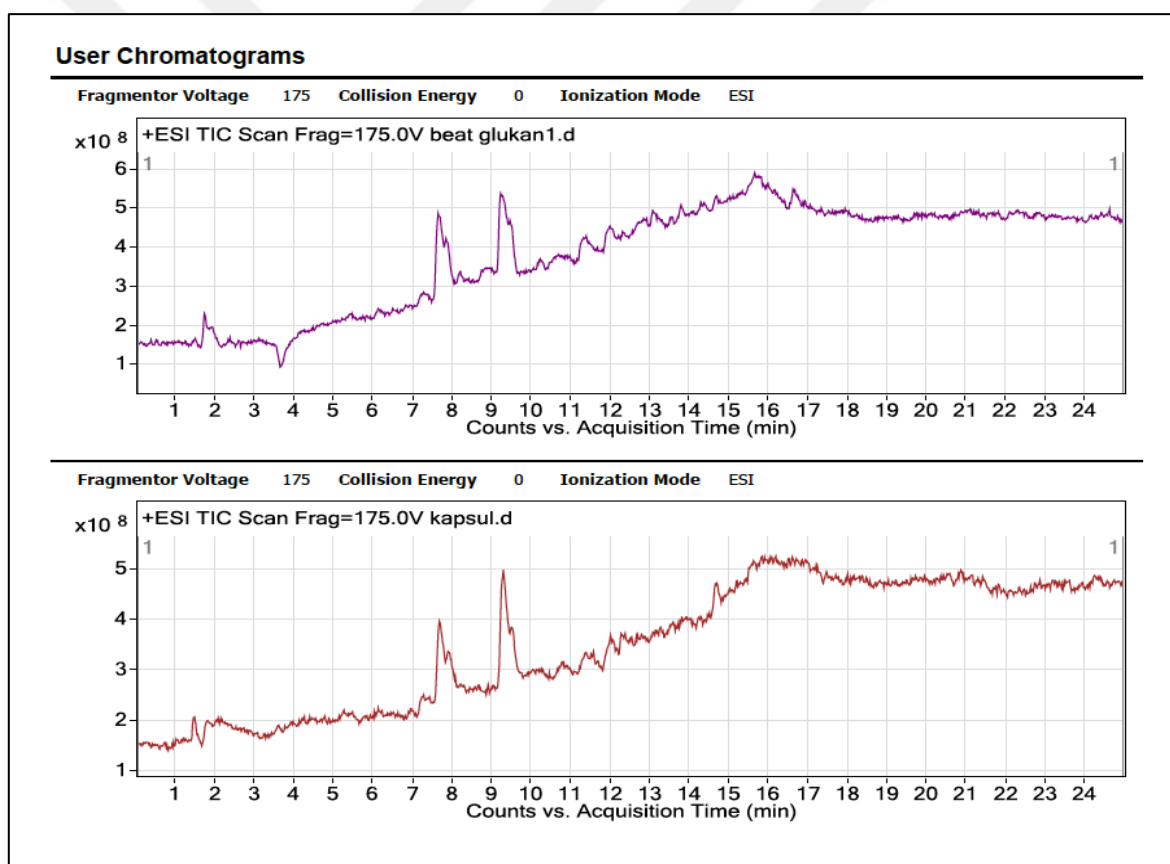


Figure 3.11. LC-MS Chromatograph Analysis sample and standard β -glucan (a)

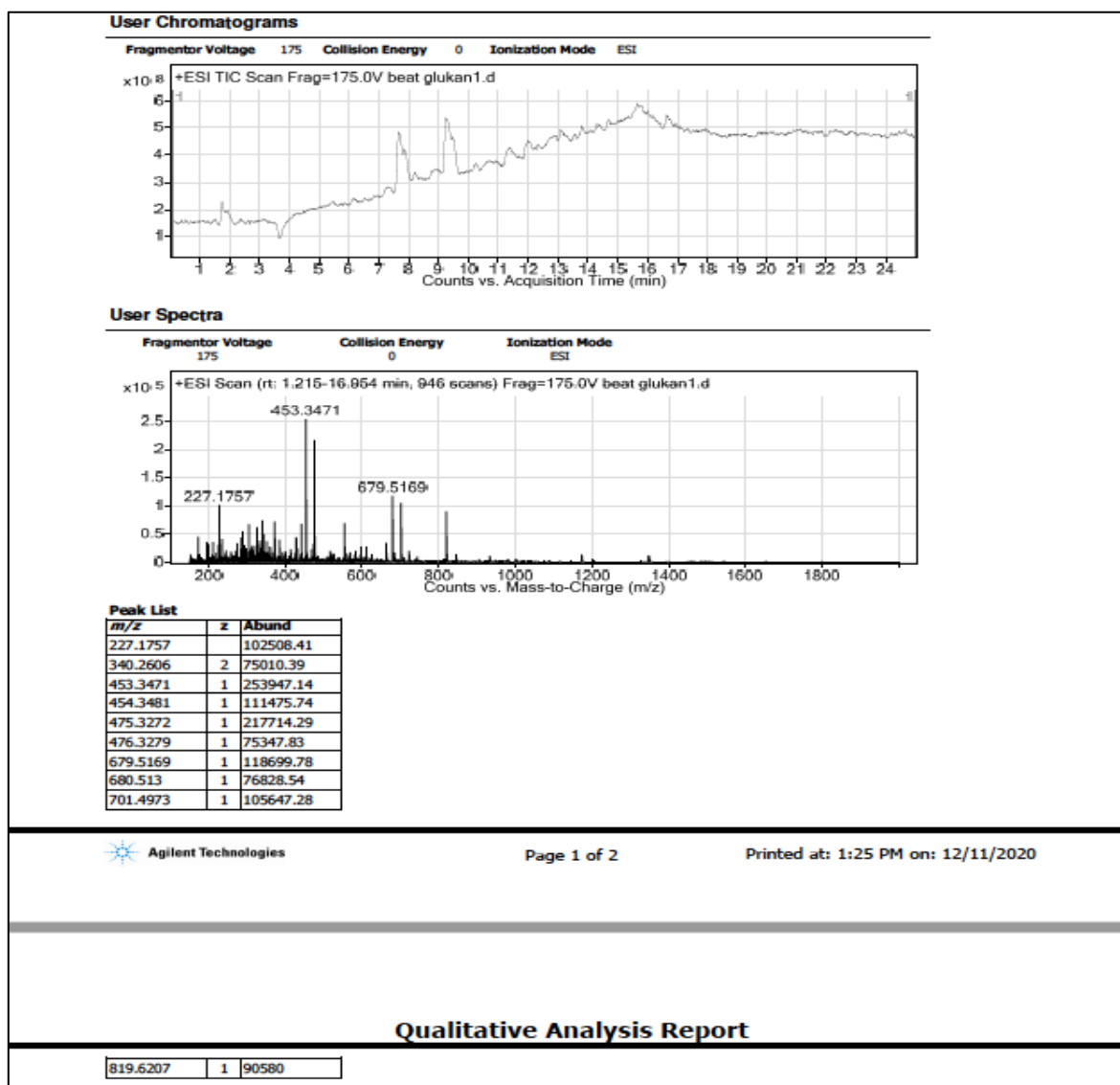


Figure 3.12. LC-MS Chromatograph Analysis sample β -glucan (b)

In our study, we found the molecular weight of β -glucan to be 349.260 g/mol. In addition, the presence of (1—6) branch chains is an important result. It increases the biological activity of β -glucan. β -glucan with a molecular weight of 349.260 g/mol has more mobility and is more able to form junctions with near chains and then easily rearranging itself. With this property, beta-glucan with 300 g/mol extensively used to prepare pseudoplastic solution in food industries [189]. In general, beta-glucan with high molecular weight is more effective in biologically in comparison to beta-glucan with low molecular weight [189]. Finding the molecular weight of β -glucan as 349.260 g/mol in our study is a good result. Another factor is the degree of branching of β -glucan that affects the biological activities of β -glucan.

Another important effect of β -glucan is its antihyperglycemic effect. The molecular weight of β -glucan adjusts the glycemia status. In one study, a beverage containing oat β -glucan (5 g) with a molecular weight of 100 g/mol provided lower postprandial glucose and insulin levels than a beverage containing barley β -glucan (5 g) with a molecular weight of 400 g/mol. In research with beta-glucan obtained from *Saccharomyces cerevisiae*, it has been shown that this product can be used as a nutraceutical as well as to lower blood sugar and reduce pain in diabetic patients [190]. Kim *et al.* (2009), showed that regularly consumed barley grains containing beta-glucan reduced insulin response [191].

In the studies conducted, β -glucan Mw ranges were found to be between 10^5 and 10^8 g/mol [192]. Commercial β -glucan had a peak molecular weight of more than 2×10^6 Daltons. Due to variations in Mw profiles and purity, the average Mw of commercial β -glucans might change greatly across batches [192,193,195]. Barley β -glucan's molecular weight varied between 0.15 and 2.5×10^6 g/mol [196, 197].

3.2.4. Antibacterial Activity

The findings of the Disk Diffusion Susceptibility Test utilizing Kirby-Bauer Disk Methods (1966)[198] were used to investigate the antibacterial activity of β -glucan against Gram-negative and Gram-positive bacteria.

Table 3.9. Antibacterial activity (Kirby-Bauer disk diffusion)

Sample/ Control	Working Rate	Zone Diameter (mm)			
		<i>P.aeruginosa</i> ATCC 9027	<i>S.aureus</i> ATCC 6538	<i>E.coli</i> ATCC 8739	<i>Enterococcus hirae</i> ATCC 10541
OFX (Standard)	100%	30	28	27	31
Beta-glucan (sample)	100%	6	6	6	6

To assess antibacterial activity, the inhibition effects of β -glucan against various microorganisms were investigated. *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, and *Enterococcus hirae* ATCC 10541 were among the bacteria found. According to Kusmiyati and Agustini (2007), the size of this zone of inhibition is brought on by the fact that Gram-positive bacteria are often more

susceptible to antimicrobial drugs than Gram-negative bacteria [199]. Amoxicillin (OFX), an antibiotic, has the ability to inhibit all known species of bacteria. It is evaluated as having extremely good inhibitory action against both Gram-positive and Gram-negative bacteria. It is recommended to use OFX since it is a broad-spectrum antibiotic, can dissolve in water, and works effectively against both aerobic and anaerobic organisms [200]. According to Table 3.5, no bacterial activity was present since none of the strains of *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, or *Enterococcus hirae* ATCC 10541 had a clean zone. The inhibition zone range of > 7 mm signifies a lack of inhibitory activity, 7-11 mm is regarded to be very weak activity, and > 16 mm is seen to be quite strong activity, according to Monks *et al.* (2002) [201]. This suggests that the absence of an inhibition zone offers a very excellent description of how all tested bacteria, both Gram positive and Gram negative, evolve, as the paper discs contain inhibitory chemicals, such as antibiotics or other antibacterial agents. According to Kusmiyati and Agustini (2007), the size of this zone of inhibition is brought on by the fact that Gram-positive bacteria are often more susceptible to antimicrobial drugs than Gram-negative bacteria [199]. The two kinds of bacteria have differing cell wall architectures, which affects how susceptible they are to antimicrobials Sudjaswadi (2006), the properties of the bacterial cell wall or wall have an effect on the action of the antimicrobial chemical [202]. The extraordinarily thick peptidoglycans that make up the cell wall of Gram-positive bacteria are what give the cells their rigidity. It is feasible to stop the formation of cross-links in the peptidoglycan cell wall and obstruct cell wall synthesis in Gram-positive bacteria by giving them antimicrobial medications. This will cause the bacteria to die. Cell lysis brought on by damage to the cell wall or impediments that prevent it from developing might result in a wider zone of inhibition [200].

Table 3.10. Antibacterial activity correlations

	Sample/ Control				t	p
	OFX (Standard)		Beta-glucan			
	X	ss	X	ss		
<i>P.aeruginosa</i> ATCC 9027	29.67	0.57	6.00	0.10	70.317	0.000
<i>S.aureus</i> ATCC 6538	27.97	0.06	5.97	0.06	440.847	0.000
<i>E.coli</i> ATCC 8739	27.04	0.06	6.00	0.10	319.161	0.000
<i>Enterococcus hirae</i> ATCC 10541	30.67	0.67	6.03	0.15	62.457	0.000

This table presents the results of an experiment conducted on four different bacterial species (*P.aeruginosa* ATCC 9027, *S.aureus* ATCC 6538, *E.coli* ATCC 8739, and *Enterococcus hirae* ATCC 10541). Two different substances named OFX (Standard) and Beta-glucan were applied to these bacteria and the results were compared. There are several important points that can be inferred from the table: For each bacterial species, there is a significant difference between OFX (Standard) and β -glucan. This is confirmed by t-test statistics and p-values. In each case, the p-value is less than 0.05, indicating that the differences are statistically significant. The difference between OFX (Standard) and Beta-glucan varies for each bacterial species. This indicates that each bacterial species responds differently to these two substances. The average measurement values differ between OFX (Standard) and Beta-glucan for each bacterial species. This indicates that the response to each substance varies depending on the bacterial species.

The standard deviation values are generally higher for Beta-glucan for each bacterial species. This indicates that the measurements obtained with Beta-glucan show greater variability. This table compares the responses of different bacterial species to OFX (Standard) and Beta-glucan substances. The results indicate that the responses to these two substances vary depending on the bacterial species, and these differences are statistically significant.

3.2.5. Rheological Properties

The shear stress (τ) versus shear rate ($\dot{\gamma}$) data for β -glucan solutions were fitted well to Herschel-Bulkley model at 25 °C (Eq. 3.1) [203].

$$\tau = \tau_0 + k (\dot{\gamma})^n \quad (3.1)$$

In the equation, shear rate is denoted by $\dot{\gamma}$ (s^{-1}), yield stress is denoted by τ_0 (Pa), consistency coefficient is denoted by k ($Pa \cdot s^n$), and flow behavior index is denoted by n .

Table 3.11. Herschel-Bulkley parameters of β -glucan at 25 °C.

Concentration (%) β -glucan	τ_0 (Pa)	k (Pa.s ⁿ)	n
0.8	$6.4122 \cdot 10^{-3}$	0.0023813	0.99751
0.6	$1.7218 \cdot 10^{-3}$	0.0020619	1.0251
0.4	cannot be calculated	0.002209	0.99957
0.2	$0.51346 \cdot 10^{-3}$	0.0020312	1.0094

For all the samples in Table 3.11, τ_0 (Pa) represents yield stress, and k (consistency coefficient) and n (flow behavior index) values were calculated with Statistica program by applying Herschel-Bulkley model. The range of the flow behavior index (n) is 0.99751 to 1.0251. The greatest n value for β -glucan concentration at 0.6% was 1.0251, indicating that it behaves like a Newtonian fluid (n=1) [203].

In β -glucan solutions, a considerable level of yield stress was seen. Yield stress is defined as the least amount of tension necessary to start a flow. This means that greater pressures are required to deform β -glucan solutions, as shown by the rise in yield stress with concentration [204]. High concentrations of water-binding macromolecules including fibers, emulsifiers, and hydrocolloids are thought to improve how well baked foods combine and extend their shelf life. The enhanced water holding capacity of β -glucan solutions may be the cause of the greatest yield stress and consistency index values [205].

Since yield stress could not be determined at each concentration, the Power-Law model was used. Power-law flow modelling of fluids; the most common approximation method used to describe the viscous flow behavior of non-Newtonian fluids (such as polymer melts) uses a Power-Law relationship.

$$\tau = K \dot{\gamma}^n \quad (\text{Ostwald de Waele model})$$

where K is the consistency coefficient(Pa.sⁿ)

n is the flow behavior index(dimensionless), $\dot{\gamma}$ (s⁻¹)=shear rate

If n=1, then K= η =viscosity

For pseudoplastic fluids, $n < 1$

For dilatant fluids, $n > 1$

Yield stress was enhanced in parallel with increase in β -glucan concentration and the highest value of yield stress was observed in 0.8 % concentration of β -glucan. Similar findings have been reported by Bhatt(1995), Dawkins and Nnanna (1995), Burkus and Temelli (2005), Colleoni-Sirghie et al., (2003) and Ghotra et al., (2009) for β -glucan of mushroom, barley bran, oat, barley and high purity barley β -glucan, respectively [206, 207, 208, 209, 210]. According to Szczesniak and Farkas (1962), the presence of yield stress in certain hydrocolloids can be attributed to intramolecular interactions. For example, the interaction of acetate groups in xanthan gum leads to the development of yield stress. In contrast, β -glucan lacks charged groups that contribute to such interactions. Therefore, the strengthening of hydrogen bonds at higher concentrations could potentially be the cause of yield stress. Yield stress is a valuable and practical property of hydrocolloids, particularly when they are employed as viscous agents in food formulations. It helps to safeguard the structure of food against stress and temperature variations [211].

Table 3.12. Effect of 0.8%, 0.6%, 0.4% and 0.2% concentrations on consistency (K), flow index(n) and correlation coefficient of Power law model(R^2)

Concentration (%) β -glucan	K(Pas ⁿ)	n	R^2	SD
0.8	7.5825×10^{-3}	0.68977	0.93467	0.015917
0.6	2.6044×10^{-3}	0.96568	0.99827	0.0025671
0.4	2.3354×10^{-3}	0.98239	0.9995	0.0013106
0.2	2.252×10^{-3}	0.98184	0.9993	0.0014983

For all the samples in Table 3.12, R^2 (coefficient of determination), K (consistency coefficient), and n (flow behavior index) values were calculated with Statistica program by applying Power-Law model. The present study was carried out to investigate flow behavior of β -glucan, as influences of concentrations (0.8%, 0.6%, 0.4% and 0.2%) temperature 25°C. Shear stress data were fitted versus shear rates using the model. Since our sample is a polymer solution, its viscosity varies depending on the shear rate. Since it does not have a fixed viscosity, it must fit a model. Shear shows thinning behavior. While it is a solid sample at rest, it becomes thinner and more liquid as the mixing or pumping process accelerates.

Shear-thinning fluids, also known as pseudoplastic fluids, exhibit a decrease in viscosity with increasing shear rate. Polymer solutions and other solutions containing high molecular weight compounds are typical examples of pseudoplastic fluids.

These fluids will develop shear stress when the shear rate is modest. The molecules reorganize as a result of the shear stress to lessen the total stress. The viscosity of polymer solutions increases as the increase of the molecular weight of the polymer increases since longer chains occupy more space and increase the resistance to flow. As a biopolymer, the molecular weight of β -glucan affects the viscosity [85]. Since the extraction method is one of the factors determining the molecular weight.

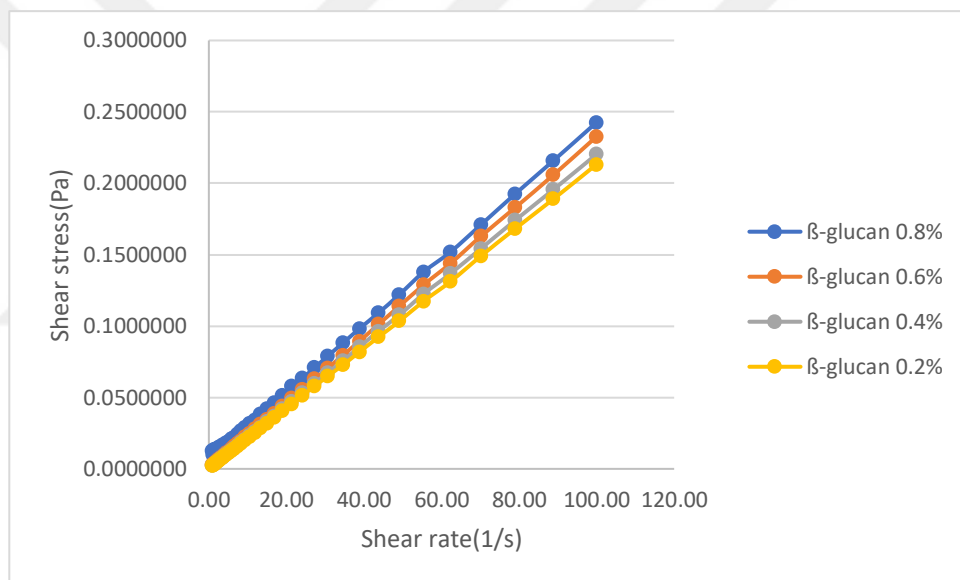


Figure 3.13. Flow curves obtained for %0.2, %0.4, %0.6, %0.8 β -glucan solution at 25°C

Figure 3.14 shows how the viscous (loss) (G'') and elastic (storage) moduli (G') work of β -glucan remedies. The findings showed that values increased in all samples with increasing frequency. These powder suspensions exhibit viscoelastic behavior, which describes their dual viscoelastic properties.

These β -glucan solutions have viscoelastic behavior, meaning they have both viscosity and elastic elasticity. All samples' elastic modulus values were discovered to be higher than their viscous modulus values, demonstrating the solid behavior of the β -glucan solutions. Moreover, the elastic and viscous moduli of β -glucan solutions were high. Greater shear rates are produced

by β -glucan solutions over longer periods of time, which leads to the formation of more hydrophilic moieties and higher modulus values. By maintaining a consistent pressure throughout the procedure, β -glucan solutions create finer particles [212].

Figure 3.14 demonstrates that every sample exhibits the same pattern. a distinction between G' and G'' when the water content rises, values fall. When dispersions are diluted with water, the sample tends to exhibit characteristics more akin to a viscous liquid rather than an elastic solid, which is in line with expectations.

In the study, an oil/water interfacial film was formed using gum arabic and modified starch and frequency shear test was performed on the film. As a result of the studies conducted at 20% and 10% concentrations of gum arabic, higher elastic properties were obtained at 20% concentration and viscous properties were found to be dominant in the 10% sample. Thus, it was stated that especially the elastic modulus properties increased as the concentration increased [212].

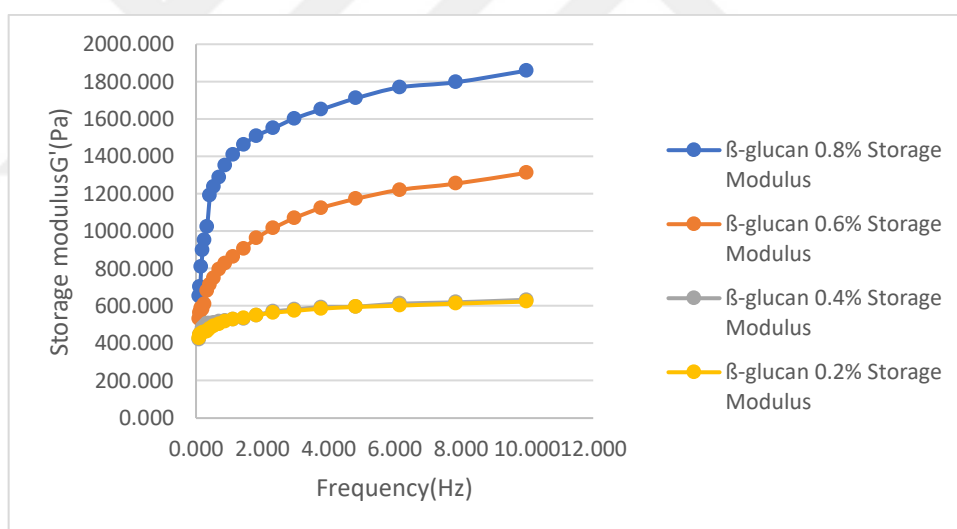


Figure 3.14. Storage modules(G') obtained for β -glucan solutions at different concentrations. G' at 0.2%, G' at 0.4%, G' at 0.6%, G' at 0.8 %solution

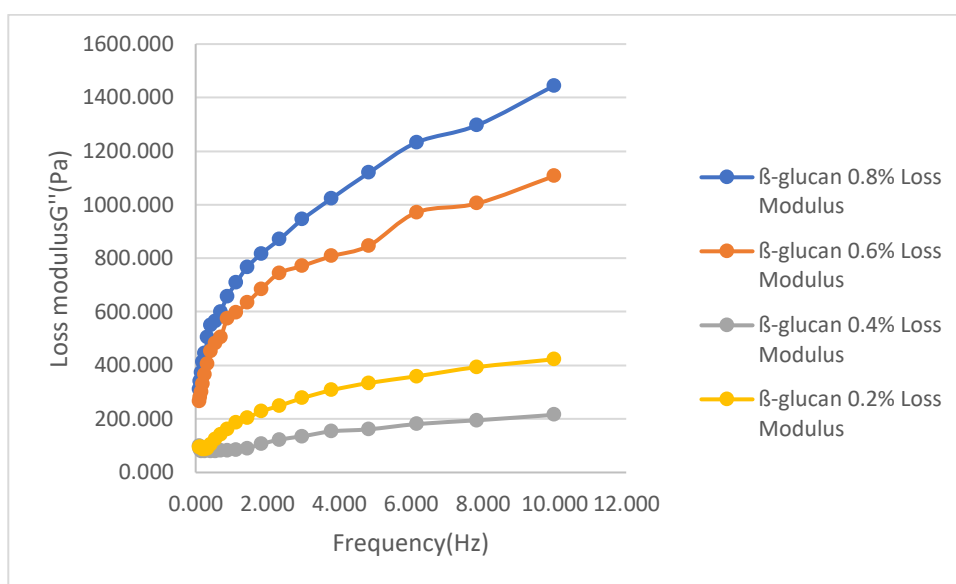


Figure 3.15. Loss modules(G'') obtained for β -glucan solutions at different concentrations.

G'' at 0.2%, G'' at 0.4%, G'' at 0.6%, G'' at 0.8 %solution

Three duplicates of the shear stress measurements for each shear rate were made, and their average results were computed. To assess whether model performs better in predicting the rheological behavior of β -glucan, R^2 and RMSE were determined. As a rule, the model with the greatest R^2 and lowest RMSE without any estimated parameter values with negative values was chosen as the best model. Power-Law was the best model for fitting shear stress data vs shear rate, despite models with yield stress having high R^2 (>0.99) and low RMSE.

The fitting of shear stress versus shear rate data by power law model indicated that β -glucan of mushroom shows non-newtonian behavior in different concentrations. Flow behavior index (n) less than 1 for all concentrations suggest pseudoplastic behavior (shear-thinning behaviour). Colleoni-Sirghie *et al.* (2003), reported that by increasing oat β -glucan concentration from 0.25 to 1%, flow behavior index was decreased from 0.855 to 0.504 [209].

Hydrocolloid solutions with high flow behavior index ($n > 0.7$) cause an unfavorable slimy feeling in the mouth and are suitable as fat replacer in foods. Therefore, for providing high viscosity and good mouthfeel, hydrocolloids with low flow behavior index should be used.

In dynamic tests, the main viscoelastic properties are the storage (G') and loss (G'') moduli which allow the calculation of $\tan \delta = G''/G'$. In this work, these properties were mainly

determined by frequency sweep tests. Temperature scanning tests were not performed because this system does not show thermal transitions.

According to Steffe (1996), materials usually exhibit more solid-like characteristics at higher frequencies [212]. This means that a G'' and G' crossover would be expected around high frequencies, as observed by Torres *et al.* studying gels based in Chitosan crosslinked with glutaraldehyde [213].

The model is a useful option for forecasting the rheological behavior of hydrocolloids because hydrocolloid components behave as scattered particles in aqueous media. The Power Law model is the most effective one for fitting shear stress data versus shear rate according to Koocheki *et al.* (2013), solution of *L.perfoliatum gum* [214].

Table 3.8 displays the parameter values for rheological models with yield stress. As anticipated, each model's β -glucan content grew along with the consistency coefficients. This result is comparable with those for xanthan gum and *L. perfoliatum* seed gum published by Koocheki *et al.* (2013). The flow behavior index may be used as a guide to show how fluids thin under shear [214].

All β -glucan solutions regardless of concentration have a shear thinning tendency, as shown in Table 3.8 and 3.9 for the Dynamic and Power law models. The flow index fell as the β -glucan concentration rose, demonstrating that the solution's pseudoplasticity was boosted by raising the β -glucan concentration. The ideal design of food processing systems may benefit from knowledge of yield stress [215].

According to Szczesniak and Farkas (2006), intramolecular interactions are the cause of yield stress in several hydrocolloids. Hence, the reason of yield stress may be the strengthening of hydrogen bonds at greater concentrations. In particular, when hydrocolloids are utilized as viscosity agents in food forms, preserving the food structure from stress and temperature change, yield stress is a valuable and practical attribute of hydrocolloids [211].

The findings demonstrated that the consistency coefficient grew non-linearly as the content of β -glucan rose. This is most likely caused by the molecules' increased ability to retain water. Increasing hydrocolloid concentration boosts intramolecular interaction and entanglement, which raises consistency coefficient and apparent viscosity. These effects are accompanied by a rise in consistency coefficient. Similar findings on the impact of

concentration on the improved consistency coefficient of salep, and *A. homolocarpum* seed gum were published by Koocheki (2013). correspondingly, *L. perfoliatum* seed gum [214].

While under stress, a material's rheological qualities lead it to flow and deform. No matter how quickly the shear rate varies, the viscosity of Newtonian fluids stays constant. Depending on the kind of fluid, the viscosity of the fluid may either grow or decrease when the shear rate is raised. The viscosity decreases as the shear rate increases for fluids that are shear thinning, but the converse is true for fluids that are shear thickening. If the force isn't great enough to break the plastic, it won't flow. Time-dependent fluids have viscosities that change over time. Foods that are both elastic and viscous are known as viscoelastic foods. Tests for dynamics, creep, and stress relaxation may all be used to find materials that have viscoelastic qualities. The characteristic resistance to motion of a fluid is referred to as its "viscosity."

Non-Newtonian fluids are fluids that do not adhere to Newton's viscosity law. Fluids that are shear thinning or thickening follow the power law paradigm (Ostwald-de Waele equation). fluids that shear thin (or are pseudo-plastic). Layer-to-layer friction in these fluids diminishes as shear rate rises. Viscosity is decreased via shearing, which forces entangled, long-chain molecules to straighten up and align with the flow. Paint is a common illustration of a fluid that shears thins.

When paint is put to a surface without using a brush, its viscosity rises and it is unable to flow due to gravity. The viscosity of paint diminishes when it is applied to a surface using a brush that shears the paint. Apple sauce, banana puree, and concentrated fruit juices are a few examples of fruit and vegetable products that are ideal examples of pseudoplastic fluids in food systems. Krokida *et al.* (2001) looked into the rheological characteristics of fruit and vegetable products. Although the flow behavior index n somewhat dropped with concentration, the consistency coefficient k grew rapidly. For pulpy goods, the flow behavior index was near to 0.5, while for clear juices, it was close to 1.0. The influence of temperature on both the apparent viscosity, and the consistency coefficient of the power law model, k , was described using an Arrhenius-type equation [215]. Nevertheless, the flow behavior index was believed to remain generally constant with temperature. Foods' rheological properties may vary as they get more concentrated. Shear thinning was seen in the rheological behavior of concentrated grape juice with a Brix value of 82.1. Yet it was

discovered that diluted samples with Brix values ranging from 52.1 to 72.9 were Newtonian. A staple food item in Turkish breakfast, sesame paste-concentrated grape juice blends were examined for their rheological behavior at two different sesame paste concentrations: 35% to 65°C and 20% to 32°C. With a flow behavior index ranging from 0.70 to 0.85, all of the mixes demonstrated shear thinning behavior [216].

The storage modulus (G') and loss modulus (G'') of the samples, respectively, were used to describe their elastic and viscous characteristics (Table 3.9). Plot the samples' loss and storage moduli vs frequency and display them. Differentiating between a solution and a gel-like structure is possible thanks to the slopes of the storage modulus (G') and the loss modulus (G''). The similar response was shown in the situations of β -glucans 0.2% and 0.4%. The samples with the greatest and lowest elasticity (G') were 0.8% and 0.2% of β -glucan, respectively. This tendency was only apparent in the samples of β -glucan 0.2%, 0.4%, 0.6%, and 0.8% in the current investigation.

In certain circumstances, a decline in the storage modulus (G') values at higher frequencies may indicate that the network structure has broken apart into chains. All samples' storage moduli (G') rose with frequency before reaching a plateau. The plateau values of G' may be utilized to determine the networks' tensile strength and elasticity.

Due to their increased β -glucan content, the samples' corresponding increases in G' may be explained. The creation of network architectures is impacted by the arabinoxylans' interference with crosslinking, aggregation processes, and intermolecular interactions between β -glucan and arabinoxylan. It has been discovered that, in certain instances, rather than the network development, the higher elasticity of samples with bigger M_w is related to the stronger entanglements of these samples [217].

According to one definition, a gel is a substance in which G' has a positive slope and $G' > G''$ at 6 Hz. [217]. The mechanical spectra of β -glucan solutions began to first adopt elastic networks ($G' > G''$) for β -glucan 0.2%, 0.4%, 0.6%, and 0.8% samples. For samples of 0.2%, β -glucan 0.4%, β -glucan 0.6%, and β -glucan 0.8%, the loss modulus response (G'') rose with increasing frequency. Although G' was always greater than G'' in the solutions of the 0.2%, β -glucan 0.4%, β -glucan 0.6%, and β -glucan 0.8% samples, these samples exhibited elastic characteristics.

Skendi *et al.* extracted isolated β -glucan from two oat varieties' ground seeds. All of the β -glucan samples tested were capable of generating gels, according to dynamic rheometry. In contrast to their counterparts with high Mw, the low Mw oat β -glucan exhibited high gelation rates and rapid gelation periods. It was previously noted that the low-Mw β -D-glucan gels up more quickly than the high-Mw β -D-glucan. Low-Mw β -glucan's high mobility and high structural regularity make junction zones and three-dimensional networks simple to form, which in turn speeds up gelation [217]. The solution becomes more viscous due to β -glucan. A distinctive quality of the polysaccharide solution is also thought to be its inherent viscosity. According to some theories, the concentration, capacity to form aggregates, and molar mass of the polysaccharides all affect viscosity. Many investigations have shown the strong relationship between the Mw of β -glucan and solution viscosity. Kim and White (2010) also noted that the viscosity increased when the Mw of oat β -glucan increased [218]. Moreover, it has been shown that gamma irradiating β -glucan causes the radiolysis of glycosidic bonds, which lowers its Mw and subsequently lowers its viscosity [218].

Viscosity; among the samples, variations in slurry viscosities were noted. Variations in polysaccharide's Mw and solubility cause variations in viscosity. Figures 3.16 show the apparent viscosities of samples containing β -glucans at various shear rates.

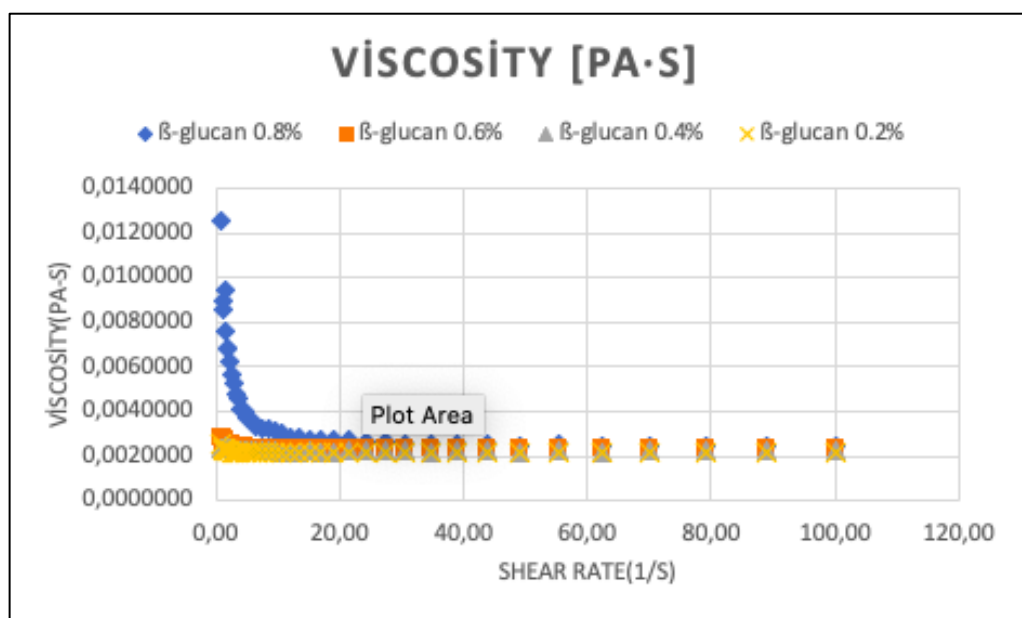


Figure 3.16. Shear rate dependence of viscosity for 0.8, 0.6, 0.4, 0.2% β -glucan solution

Shear thinning characteristics are also primarily influenced by variations in Mw and the fine structure of β -glucan. The viscosity of each sample usually decreased as the shear rate increased. The shear applied causes the molecular entanglements of the β -glucan to be broken, which results in the shear thinning flow characteristic. According to reports, Mw influences when shear thinning behavior starts.

According to Lazaridou *et al.* (2007), the β -glucans solutions exhibit high apparent viscosities at low shear rates and a shear thinning flow at high shear rates. Smaller polymers have been suggested to increase the degree of aggregation because of their higher concentrations [219]. The rate of diffusion may increase viscosity. As β -glucan competes with starch for water [220], Faraj *et al.* (2006) found that the purity of the β -glucan was a key factor in the viscosity of β -glucan starch blends [221]. Nevertheless, Papageorgiou *et al.* (2005) found a modest correlation between total β -glucan concentration and slurry viscosity [220].

3.3. PRODUCTION OF CRACKERS

The formulation and baking process used in the production of crackers were applied by modifying the method specified in the American Association of Cereal Chemists (AACC 2017) [222] standard. The amount of wheat flour used in this formulation was reduced by 5, 10, 15, 20, 25, and 30g β -glucan was used instead, and the other ingredients and their amounts in the formulation were not changed (Figure 3.17).





Figure 3.17. Crackers with varying amounts of β -glucan

3.3.1. Chemical Analyses Performed in Crackers

Results of analysis are shown in Table 3.17.

Table 3.13. β -glucan amount (g), protein (%), ash (%), moisture (%)

Samples	β -Glucan amount(g)	Protein(%)	Ash(%)	Moisture(%)
1	0	9.40 $\pm$ 0.23	2.10 $\pm$ 0.06	4.15 $\pm$ 0.21
2	5	9.45 $\pm$ 0.23	2.08 $\pm$ 0.06	5.07 $\pm$ 0.21
3	10	9.58 $\pm$ 0.23	2.33 $\pm$ 0.07	5.27 $\pm$ 0.23
4	15	10.50 $\pm$ 0.26	2.24 $\pm$ 0.06	6.06 $\pm$ 0.23
5	20	10.67 $\pm$ 0.26	2.25 $\pm$ 0.06	6.21 $\pm$ 0.21
6	25	10.72 $\pm$ 0.26	2.74 $\pm$ 0.02	6.63 $\pm$ 0.22
7	30	10.93 $\pm$ 0.27	2.76 $\pm$ 0.02	7.74 $\pm$ 0.23

The protein value (%) decreases as the amount of β -glucan (g) approaches to 0 while the protein value (%) is the highest in those with a β -glucan amount (g) of 30. While the protein (%) value is close to each other in which the amount of β -glucan (g) is between 0-10, the difference increases more when the amount of β -glucan (g) is 15.

The moisture value (%) decreases as the amount of β -glucan (g) approaches to 0 while the moisture value (%) is at its highest level in those with a β -glucan amount (g) of 30. Increases in the moisture value (%) are greater when the amount of β -glucan (g) changes from 0 to 5, 10 to 15, and 25 to 30.

The ash value (%) of those with β -glucan (g) amount of 30 is the highest, while the ash value is lowest when the amount of β -glucan (g) is 5. Although a decrease is observed in the ash value (%) when the amount of β -glucan (g) changes from 0 to 5 and 10 to 15, the ash value (%) increases in all other changes of the amount of β -glucan (g). The amount of increase is greatest when the amount of β -glucan (g) changes from 20 to 25.

As the amount of β -glucan (g) increases from 0 to 30, the protein (%) and moisture (%) values increase proportionally. On the other hand, On the other hand, there is a decrease in Ash (%) values at 0-5 and 10-15 changes despite of a proportional increase in all other changes.

Table 3.14. Statistical analysis showing the relationship of β -glucan amount(g) with protein (%), ash(%) and moisture(%)

		Beta-Glucan amount (g)	Protein (%)	Ash (%)	Moisture (%)
Beta-Glucan amount (g)	r	1	.946**	.880**	.980**
	p		.001	.009	.000
	n	7	7	7	7
Protein (%)	r		1	.738	.921**
	p			.058	.003
	n		7	7	7
Ash (%)	r			1	.842*
	p				.017
	n			7	7
Moisture (%)	r				1
	p				
	n				7

*p<0,05 **p<0,01

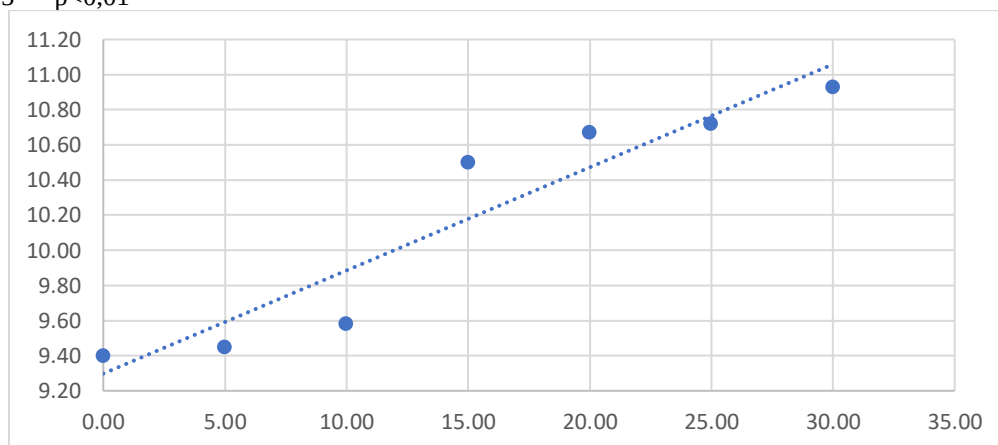


Figure 3.18. Relationship between β -Glucan amount (g) and Protein (%)

According to the results of the analysis, there is a positive and powerful relationship between β -Glucan amount (g) and Protein (%) ($r=0.946$; $p<0.05$). Accordingly, when the β -glucan amount (g) value is high, the protein (%) value is also high.

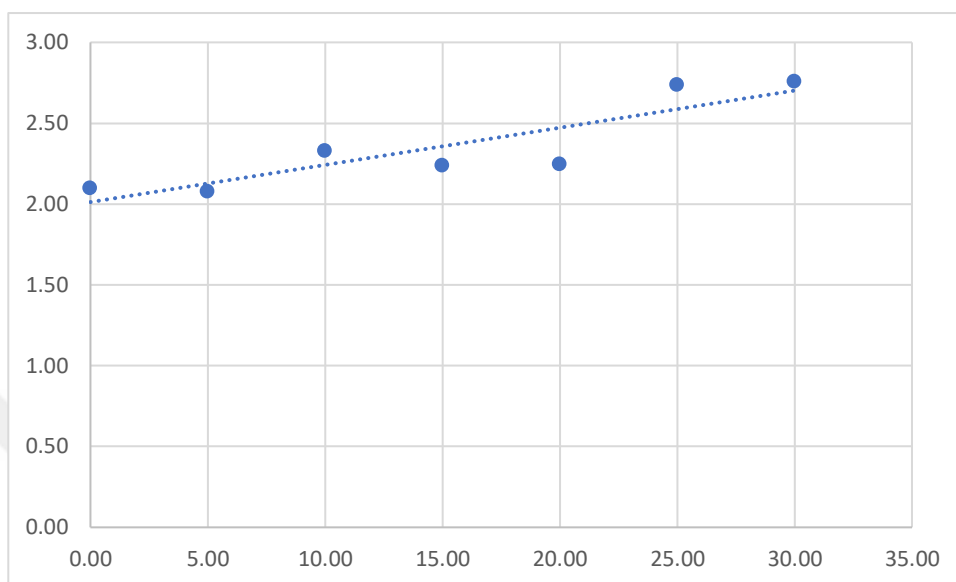


Figure 3.19. Relationship between β -Glucan amount (g) and Ash (%)

There is a positive and powerful correlation between β -glucan amount (g) and ash (%) ($r=0.880$; $p<0.05$). Accordingly, when the β -glucan amount (g) value is high, the ash (%) value is also high.

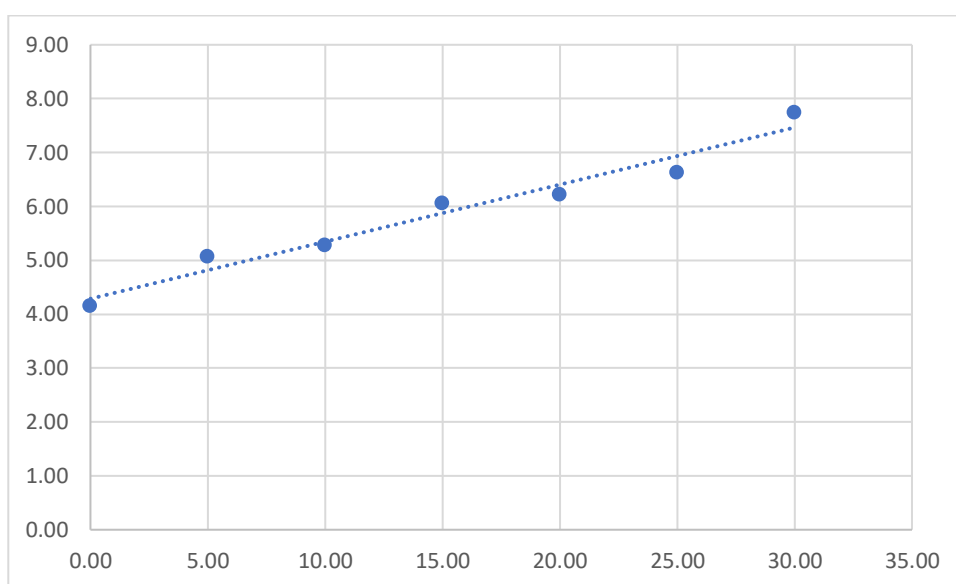


Figure 3.20. Relationship between β -Glucan amount (g) and Moisture (%)

There is a positive and powerful relationship between β -glucan amount (g) and moisture (%) ($r=0.980$; $p<0.95$). Accordingly, when the β -glucan amount (g) value is high, the moisture (%) value is also high.

The moisture content of crackers is sensitive to temperature fluctuations in the oven. The moisture level primarily affects the shelf life of the biscuit. The baking process is the transformation of the viscoelastic dough into a solid baked product. This process determines the physical properties of the final biscuit, including cracker dimensions (diameter and thickness), biscuit weight and biscuit moisture content [223].

In order to eliminate the temperature effect in the analysis, crackers were baked at the same temperature and time. In terms of moisture content, the samples were not statistically very different from each other, and the moisture content increased in the biscuits with high levels of β -glucan. It was considered that moisture retention in the samples to which β -glucan, which has a fibrous structure, was added could lead to this situation. Hydrocolloids are known to be used as water retention capacity enhancers. In a study, 10% and 20% β -glucan was added to oat biscuits, which increased the moisture content and stickiness of the biscuit and improved its taste [224]. Oat flour added to bread making increased the water content of the breads as it retained more water in the dough than the control sample due to its fibrous material [225]. The increase in ash content in the samples to which β -glucan was added was found to be significant, and it was thought that this may be due to the ash content of the β -glucan added. The quality of the biscuit depends on the physicochemical properties of the dough. Flour, fat and water levels and cooking parameters also affect protein composition. Biochemical and physicochemical reactions that occur during the baking process (such as water evaporation, protein denaturation and Maillard reaction) affect biscuit quality [225].

The 30% β -glucan content with the highest protein content was found to be significantly different. The addition of high amounts of β -glucan did not decrease the diameter values of the crackers, but did not increase them significantly.

3.3.2. Textural Properties of Craker Samples

Texture properties, which are important parameters for the acceptance of food by consumers, determine the production capacity. The texture of bakery products such as crackers is

typically characterized by its mechanical properties. Table 3.19 shows the hardness values of these doped crackers. Hydrocolloids have a weakening effect on the structure of starch. Some studies have suggested that the increase in dough softness is parallel with the increase in the concentration of hydrocolloids. Hardness refers to the force required to compress the material by a given amount. Fracturability refers to the ease with which the material will break.



Figure 3.21. Texture analyser

Table 3.15. Structure analysis of cracker samples produced with the addition in different amounts of β -glucan (g)

Sample	Dry Matter(%)	Hardness (g-force)	Fracturability(g-force)
Control	95.85	17 126.45 $\pm$ 2345.32	17 255 $\pm$ 2432.12
5 g β -glucan	94.93	17 852.47 $\pm$ 3437.58	13 906.85 $\pm$ 2674.58
10 g β -glucan	94.73	17 814.07 $\pm$ 4180.41	13 082.46 $\pm$ 1811.05
15 g β -glucan	93.94	31 169.05 $\pm$ 3797.95	13 434.02 $\pm$ 2702.84
20 g β -glucan	93.79	34 129.30 $\pm$ 4443.23	7584.98 $\pm$ 985.67
25 g β -glucan	93.37	16 886.23 $\pm$ 3105.48	16 538.52 $\pm$ 1878.92
30 g β -glucan	92.26	15 409.50 $\pm$ 1306.27	10 936.43 $\pm$ 1629.09

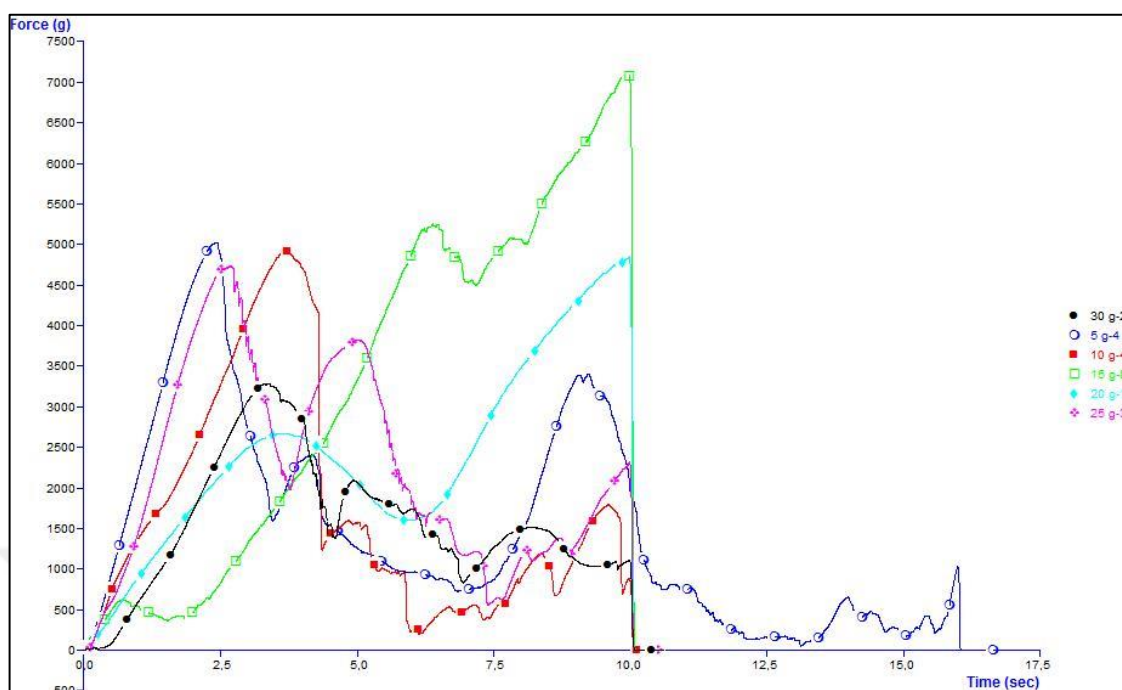


Figure 3.22. Graphic of the crackers hardness (g-force) and fracturability(g-force)

The maximum breaking force (N) values of β -glucan added crackers were found to be lower than the control. As the β -glucan content added to the crackers increased, the hardness decreased. It is thought that this decrease in the hardness of the crackers may be due to the dilution of the protein gluten with the addition of β -glucan and thus the delay in the formation of the gluten matrix, β -glucan absorbs water, and there is no free water left to be absorbed by the proteins. The cracker should have a certain crispness and hardness.

Table 3.16. Statistical analysis showing the relationship of β -glucan amount(g) with hardness(g-force), fracturability(g-force)

		Dry Matter (%)	Hardness (g)	Fracturability(g)
Dry Matter(%)	r	1	-0.072	0.212
	p		0.878	0.649
Hardness (g)	r		1	-0.564
	p			0.187
Fracturability (g)	r			1
	p			

The results of the correlation test performed to examine the relationship between Dry matter(%), Hardness (g-force) and Fracturability (g-force) are given in the Table 3.20.

According to the analysis results, there is no statistically significant relationship between Dry matter(%), Hardness and Fracturability (g-force) ($p>0.05$). However, there is no statistically significant relationship between Hardness and Fracturability ($p>0.05$).

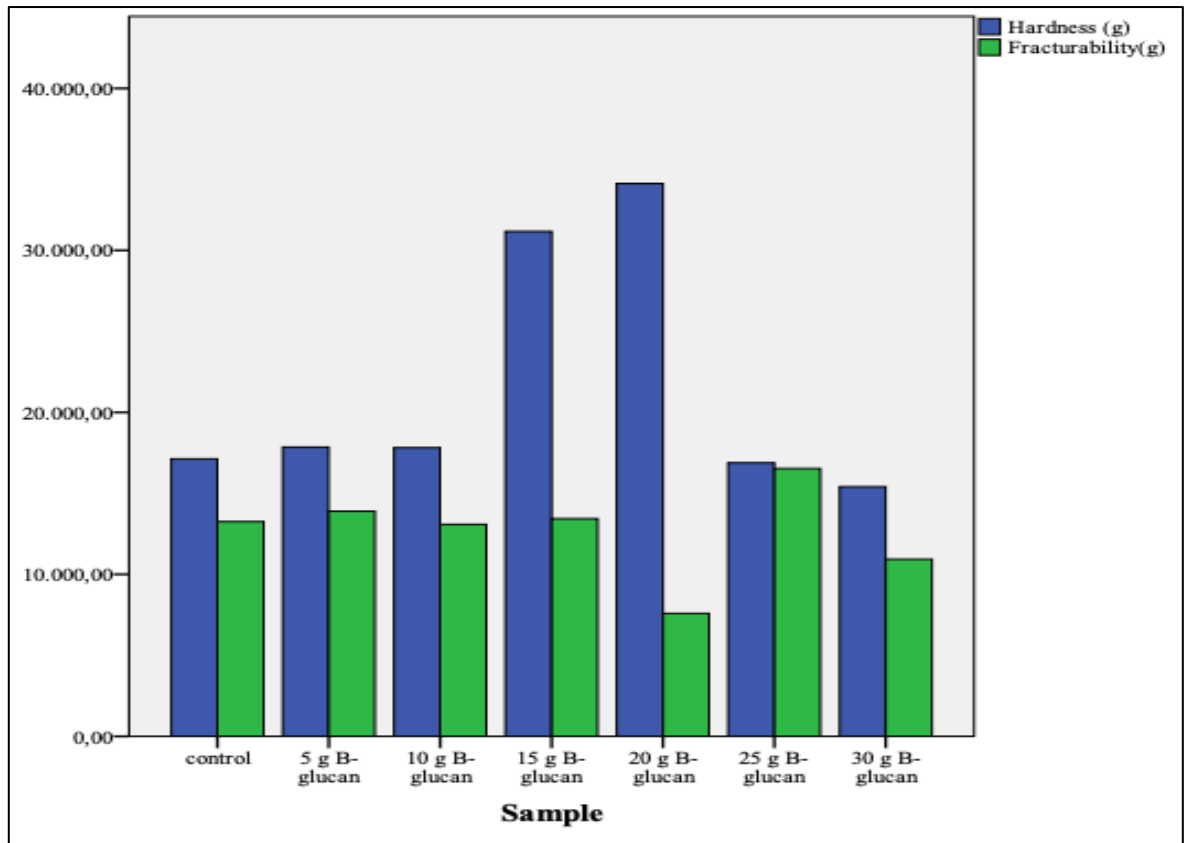


Figure 3.23. Samples (g-glucan), Hardness(g-force) and Fracturability (g-force)

According to the results of the analysis, there is a difference between Hardness(g-force) and Fracturability (g-force) in all of the samples and Hardness values are higher in each example. However, the sample where the difference is greatest is 15 g β -glucan and 20 g β -glucan.

Table 3.17. Hardness (g-force) and Fracturability(g-force) correlation analysis with sample

		Sample
Hardness (g)	r	,204
	p	,661
Fracturability(g)	r	,000
	p	1,000

In the Spearman rank correlation test, the control group was defined as 0 and the β -glucan samples were defined as 1,2,3,4,5,6, respectively. In the analysis, the relationship between the sample order and the order of the Hardness(g-force) and Fracturability (g-force) values was examined, and accordingly, no statistically significant relationship was found ($p>0.05$).

An increase in the hardness (Strength) values was observed in 15-20% cracker samples prepared by adding β -glucan at different rates, and harder and more durable crackers were obtained. It is thought that the increase in hardness is due to the increase in water absorption. The hardness value of crackers, like other quality characteristics, is affected by the dough content and the production process. In addition, hardness is one of the most important quality criteria affecting consumer taste, and a cracker that is too hard or too soft is not preferred. Excessive crisp-fragile structure causes crackers to crumble during packaging, transportation, and marketing, resulting in economic losses. The cracker should be hard enough to provide crispness and softness enough to provide an orally disintegrating texture in the mouth. Friability is an independent characteristic related to hardness and palatability, while there is an inverse relationship between hardness and dispersibility. Cracker hardness is the result of a relationship between protein and starch via hydrogen bonds. According to another explanation, cracker hardness depends on the structure of the composite matrix, which is composed of some ungelatinized starch granules, protein aggregates, and lipids. During baking, in the presence of limited water, the starch granules remain in their natural form, resulting in a crisp texture. Gluten is the source of the continuous protein matrix structure. Therefore, the gluten content of the cracker dough has a great influence on the formation of the composite matrix structure of the dough. One study reported that the properties of the matrix of crackers are primarily dependent on air gaps and fat globules [226].

When mixed with cellulose, water retention of β -glucan is higher. Vegetable fibers contain a high proportion of soluble fractions and therefore have a high-water retention capacity. Water absorption is increased due to H-bonds formed as a result of the interaction of -OH groups in the structure of dietary fiber components with water. Other ingredients in the dough composition are not properly hydrated due to the high-water absorption of dietary fiber. Hoskeney and Rogers (1994) reported that the hardness of cracker is caused by the interaction between protein and starch via the H-bond formation [227].

Türksoy (2011) observed an increase in fracture strengths in biscuits produced by adding various fruit and vegetable fibers to the biscuits in which fibers obtained from pumpkin, carrot and orange were used [228]. In similar studies, it was observed that additions such as lemon, orange pulp, apple powder caused an increase in biscuit hardness [218, 219, 220]. In the study of Fradinho *et al.* (2015), an increase in firmness value was observed as the fiber ratio increased in biscuit dough to which psyllium fiber was added. However, in present study, since the flour displacement method was not applied while adding fiber, the amount of flour did not decrease, and the firmness increased in parallel. In the study, they explained the hardening of the structure due to the interaction between proteins and carbohydrates due to the components in the added Psyllium content [229].

In the crackers obtained, the cracker with 20% β -glucan was observed to be the hardest. It is thought that the reason for the decrease in hardness value that occurs with the decrease in wheat flour ratio as a result of 30% β -glucan addition in cracker production may be related to the decrease in protein and damaged starch levels [230, 231, 232].

High viscosity soluble fibers absorb water quickly during the kneading process and utilize the water necessary for the formation of a dough with the appropriate character. The resulting gluten network can cause an increase in the hardness of the biscuit and also have a negative impact on biscuit quality. In this respect, increasing the water content of fiber-enriched doughs is important [233]. β -glucan is added as a thickening agent with its viscosity increasing properties, as a fat substitute, as a source of dietary fiber and to improve rheological properties. In a study in which the volume, textural and sensory properties of milk bread were improved by the addition of β -glucans, it was found that the viscosity improvement of β -glucans varied depending on the amount of polysaccharide dissolved from the food matrix and the molecular weight [234].

3.3.3. Hydrolysis Index and Estimated Glycemic Index

It is shown how to easily test the rate of hydrolysis of β -glucan crackers using a simple *in vitro* approach. Estimating the metabolic glycemic response to β -glucan crackers may be beneficial.

It is crucial that the *in vitro* approach enables us to as closely replicate enzymatic digestion as feasible. Alpha-amylase was utilized to hydrolyze starch in the process described in this work, and pepsin was employed to prevent protein-starch interactions. Amyloglucosidase was then used to liberate glucose from the glucose hydrolysis products. The kind of enzyme employed varies across the approaches presently being offered. As a result, although amylase is sometimes employed alone, it is also sometimes coupled with proteases [235].

We draw the conclusion that the *in vitro* approach could be helpful in GI assessment. Also, the mathematical first-order equation for each food item's hydrolysis property was found. In heated cracker slurries, GI was inversely linked with β -glucan concentrations.



Figure 3.24. UV-Spectrophotometer

The HI of the samples was calculated by comparing the area under the hydrolysis curve from the sample (0 to 120 min) with the glucose area (reference sample). The GI of the samples was calculated by using the formula proposed by Goni *et al.* [236]. Table 3.11 and Table 3.12 show the HI and *in vitro* GI values of β -glucan crackers calculated. Figure 3.43. shows

the hydrolysis curves for β -glucan crackers. The crackers with the lowest GI contained the highest amount of β -glucan compared to the other samples.

Hence, it is assumed that GI is impacted by β -glucan content. The ratio of area under the curve (AUC) in response to a test and reference meal ingested by a person on many days and under predetermined circumstances is used to calculate GI. AUC serves as a measure of blood sugar response [237]. In this context, low, medium, and high GI meals, respectively, are those with GI values between 55 and 69, over 70, and below 55 [238]. White wheat bread has a GI rating of 100 [238]. Those who are seeking to reduce their blood glucose level, such as diabetic patients, prefer to consume low GI foods. The graphs reveal a first section where the rate of hydrolysis rises between 0 and 90 minutes, and a second section where a myxial plateau is gradually attained between 90 and 120 minutes.

Table 3.18. Hydrolysis index of 5g, 10g, 15g, 20g, 25g, 30g β -glucan-containing crackers and glucose between 0 and 120 minutes

β-glucan	5 g	10 g	15 g	20 g	25 g	30 g
Time(min)	HI	HI	HI	HI	HI	HI
0	0	0	0	0	0	0
20	56,6	64,6	52,3	45,2	40,5	30,2
30	63,2	67,3	57,5	40,5	47	28,3
60	76,4	75,2	65,2	57,2	53,2	40,4
90	87,4	85,2	74,6	62,1	60,2	42,4
120	88,1	86,7	78,2	68,1	59	50,3

Table 3.19. β -glucan and HI correlation

		Time
β -glucan (5 g)	r	.835**
	p	0,000
	n	18
β -glucan (10 g)	r	.776**
	p	0,000
	n	18
β -glucan (15 g)	r	.815**
	p	0,000
	n	18
β -glucan (20 g)	r	.848**
	p	0,000
	n	18
β -glucan (25 g)	r	.805**
	p	0,000

	n	18
β -glucan (30 g)	r	.877**
	p	0,000
	n	18

This table presents the Pearson correlation coefficients (r) and associated p-values showing the relationship between different amounts of beta-glucan (5g, 10g, 15g, 20g, 25g and 30g) and time. n represents the sample size for each correlation coefficient (18). For β -glucan (5 g): $r = 0.835$, $p = 0.000$. This indicates a strong and statistically significant positive relationship between β -glucan and time. The greater the amount of beta-glucan, the greater the time. For β -glucan (10 g): $r = 0.776$, $p = 0.000$. Again, this correlation coefficient indicates a strong and statistically significant positive relationship between β -glucan and time. Similarly, there is a strong and statistically significant positive relationship with time for higher amounts of β -glucan (15 g, 20 g, 25 g and 30 g). In any case, these results are statistically significant as the p-value is much lower than 0.05. Two asterisks (**) generally indicate that the p-value is less than 0.01, representing a very strong level of statistical significance.

In conclusion, this table shows that as the amount of beta-glucan increases, the time also increases. This indicates that beta-glucan may have various applications depending on the type of process it affects.

Table 3.20. Glycemic index of 5g, 10g, 15g, 20g, 25g, 30g β -glucan-containing crackers and glucose between 0 and 120 minutes

β -glucan	5 gr	10 gr	15 gr	20 gr	25 gr	30 gr
Time(min)	GI	GI	GI	GI	GI	GI
0	0	0	0	0	0	0
20	70.78	75.18	68.42	64.52	61.94	56.28
30	74.4	76.65	63.16	61.94	65.51	55.24
60	80.96	80.99	74.46	71.11	68.91	61.72
90	87.69	86.48	80.66	73.8	72.75	62.98
120	88.07	87.31	82.64	77.09	72.1	67.32

Table 3.21. β -glucan and GI correlation

		Time
β -glucan (5 g)	r	.729**
	p	0.001
	n	18
β -glucan (10 g)	r	.690**
	p	0.002
	n	18
β -glucan (15 g)	r	.740**
	p	0.000
	n	18
β -glucan (20 g)	r	.727**
	p	0.001
	n	18
β -glucan (25 g)	r	.679**
	p	0.002
	n	18
β -glucan (30 g)	r	.716**
	p	0.001
	n	18

This table presents the Pearson correlation coefficients (r) and associated p-values between different amounts of β -glucan (5g, 10g, 15g, 20g, 25g and 30g) and time. n represents the sample size for each correlation coefficient (18).

For β -glucan (5 g): $r = 0.729$, $p = 0.001$. This indicates a strong and statistically significant positive relationship between time and β -glucan. As the amount of beta-glucan increases, the time also increases. For β -glucan (10 g): $r = 0.690$, $p = 0.002$. This correlation coefficient also shows that there is a strong and statistically significant positive relationship between time and β -glucan.

Similarly, for other amounts of β -glucan (15 g, 20 g, 25 g and 30 g), there are strong and statistically significant positive relationships between time and β -glucan.

In any case, these results are statistically significant as the p-value is much lower than 0.05. Two asterisks (**) generally indicate that the p-value is less than 0.01, representing a very strong level of statistical significance.

As a result, this table shows that as the amount of β -glucan increases, the time also increases. This may indicate that any process or event affected by β -glucan takes longer as the amount of β -glucan increases.

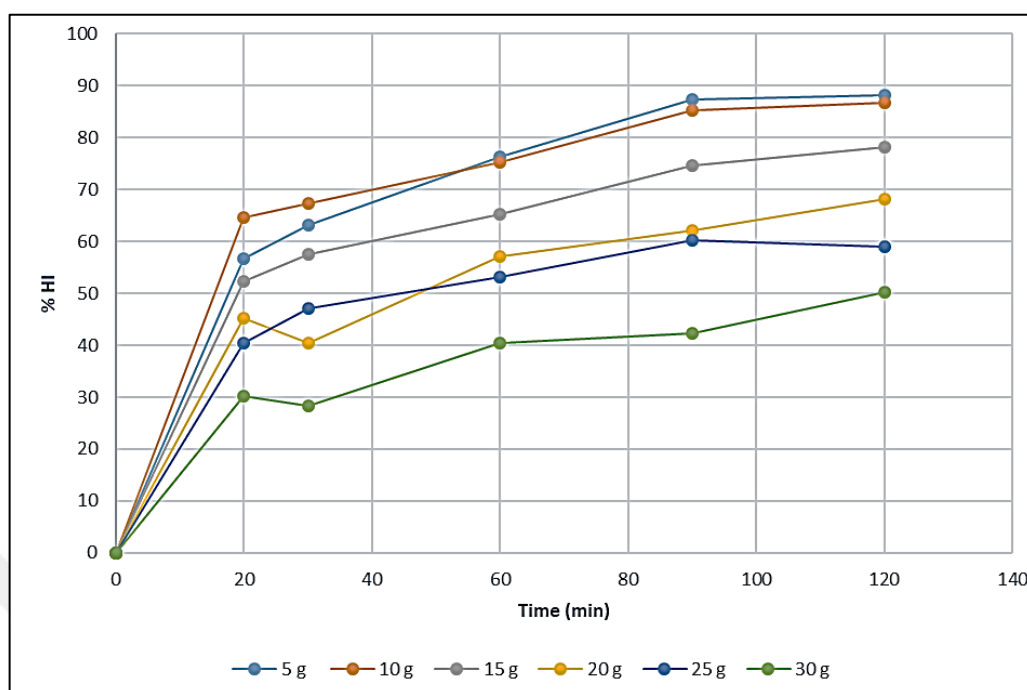


Figure 3.25. GI comparison of crackers containing β -glucan

Table 3.22. Hydrolysis index and Glycemic index correlation analysis with sample

Correlations							
		GI 5gr	GI 10gr	GI 15gr	GI 20gr	GI 25gr	GI 30gr
HI 5gr	r	,982**					
	p	,000					
HI 10gr	r		,991**				
	p		,000				
HI 15gr	r			,987**			
	p			,000			
HI 20gr	r				,972**		
	p				,001		
HI 25gr	r					,982**	
	p					,000	
HI 30gr	r						,955**
	p						,003

The highest hydrolysis index (HI) of 88.1% was observed in the 5g β -glucan cracker, resulting in a higher predicted glycemic index (pGI) of 88.07% for 5g β -glucan.

In 30 g of β -glucan, the lowest HI (30.2%) and pGI (56.38%) values were found. The findings demonstrated that the amount of β -glucan in cracker formulations correlated with reduced HI, which in turn decreased the anticipated glycemic index (pGI). A low-GI diet not

only improves certain metabolic effects of insulin resistance, but also decreases insulin resistance, according to studies [239]. Improvements in fibrinolytic activity may have a favorable role in the management of diabetes and cardiovascular disease, in addition to changes in glucose and lipid metabolism [240, 241]. Slow-digesting starch is the starch fraction that is digested slowly but not fully in the large intestine, while fast-digesting starch produces a quick rise in blood glucose levels after consumption [242]. β -glucan in crackers can be classified as a low glycemic index food, it is mainly a high-fiber food with high levels of soluble fiber. Rapid digestion and absorption of high GI meals results in noticeable changes in blood glucose levels and glucose response demand. Low GI meals are those that digest and absorb slowly, raising blood sugar and insulin levels gradually. In persons with diabetes (Type I and Type II), a low GI diet may improve glucose and lipid levels, lower insulin levels, and reduce insulin resistance, according to mounting data [243].

Jenkins *et al.* (1988), in their study, found a GI of red lentils of 37 ± 6 , consistent with the results of this study [244]. The fiber content of a food affects its glycemic index. Water-soluble fiber types such as β -glucans, pectins and gums, which increase the viscosity of the system it is in, decrease the GI value by slowing the passage of food from the stomach to the small intestine and hindering the access of enzymes to the substrate.

In a study, the GI values of red lentils and pasta were found to be lower than the GI values of rice flour. The physical structure of the food is also a parameter that affects the GI value. Dense foods such as nuts, pasta, legumes and viscous foods are digested more slowly. Thus, the release of glucose into the bloodstream slows down, resulting in a low GI. In non-viscous liquids or foods such as bread, amylase breaks down carbohydrates and, as a result, glucose quickly enters the bloodstream.

There are many factors that affect the rate of absorption of the food and carbohydrate content, and these also affect the GI value of the food. These factors include the rate of digestion, transit time, structure, preparation method, maturity, protein and fat content, quantity and type of fiber, the proportion of amylose and amylopectin in the food structure, monosaccharide components, and non-nutrient compounds such as α -amylase inhibitors [245]. Brennan and Brennan (2009) demonstrated that increasing the amount of slowly digestible starch in processed foods can reduce the glycemic index [246].

Foster-Powell *et al.* (2002) reported that the GI values of oatmeal and form biscuits were between 57 ± 3 and 70 ± 3 . Foster-Powell *et al.* (2002)'s study supports the GI results in the range of 59.1-69.9 in this study. In addition, the reported GI value of 57 ± 4 oat biscuits is quite consistent with the GI result of β -glucan crackers in this study [247].

3.3.4. Sensory Analysis

Hedonic test results are shown below. According to the hedonic test results, taste was strongly related to the overall acceptance of the β -glucan crackers. This has implications for guiding product manufacturers towards acceptable flavor enhancement approaches to promote consumer acceptance of β -glucan crackers. Differently flavored or formulated crackers should be considered for future evaluations to obtain improved sensory acceptability scores. Considering that the consumption of bagel in Turkey reaches approximately 2.5 million per day, improving the formulation and presenting it to people will increase the β -glucan utilization rate [242].

Table 3.23. Results of hedonic test

Hedonic scale	Frequency(control)	Frequency(20% beta-glucan)
Like extremely		
Like very much	1	
Like moderately	3	3
Like slightly	6	3
Never like nor dislike	6	9
Dislike slightly	2	10
Dislike moderately	7	3
Dislike very much	13	4
Dislike extremely	2	8
Toplam	40	40

During the sensory analyses, participants reported that 20% of the β -glucan samples had a slight bitter taste and if this taste was improved, the crackers could be consumed. It is predicted that this situation can be eliminated by using lower proportions of the bitter sample, and it is believed that studies on this issue and other nutritional properties of enriched β -glucan crackers should be conducted.

3.4. MATHEMATICAL MODEL FOR CALCULATING THE VALUE OF DYNAMIC VISCOSITY OF β -GLUCAN

The research work under consideration presents a detailed investigation into the rheological behavior of β -glucan solutions. It addresses key variables such as concentration, shear rate, and molecular weight, and explores their interactions to determine their influence on the solution's viscosity.

The work focuses on the characterization of β -glucan solutions. The results illustrate that the viscosity and consistency of the solutions are strongly dependent on the concentration of β -glucan. It has been shown that as the concentration increases, the ability of β -glucan molecules to retain water and the extent of intramolecular interactions also increases. These changes subsequently lead to an increase in the apparent viscosity and consistency coefficients of the solution. This finding is valuable as it allows for predictions of the solution's behavior at varying concentrations and may guide formulation strategies in industrial applications.

Shear thinning behavior, a characteristic typical of non-Newtonian fluids, is noted across all concentrations of β -glucan solutions. The study provides evidence that the pseudoplasticity of the solution is increased with the increasing concentration of β -glucan. This behavior can be valuable in contexts where solutions are subjected to varying shear rates, such as in pumping or mixing processes in industrial applications.

The research also delve into the influence of molecular weight, illustrating that variations in molecular weight and the fine structure of β -glucan significantly influence its viscosity. While not explicitly explored, the role of temperature and presence of other substances are noted as influential factors. The research explore the Power Law model and find that it is a good fit for predicting shear stress against shear rate, showing a high R^2 value and a low root-mean-square error (RMSE). This reaffirms the value of this model for systems behaving as dispersed particles in aqueous media, such as hydrocolloids.

The work culminates in the proposal of a new mathematical model, incorporating the β -glucan concentration, shear rate, and molecular weight to provide a more comprehensive description of the solution's behavior. In summary, this study provides significant insight

into the behavior of β -glucan solutions under various conditions and presents an advanced model for predicting this behavior. However, for a more comprehensive understanding, further research could be conducted exploring the role of temperature and the presence of other substances in the β -glucan solution. Additional validation of the proposed model is also recommended, potentially expanding its application to a broader range of conditions and substances. This work, nonetheless, provides a substantial foundation for understanding and predicting the behavior of β -glucan solutions, which can be invaluable in diverse fields such as food science, pharmaceuticals, and cosmetics.

The study provides a deep understanding of the rheological properties of β -glucan solutions at varying concentrations. The rheological properties of a material describe its deformation and flow characteristics under applied forces. In the case of β -glucan solutions, these properties are crucial in understanding their behavior under different conditions, which has significant implications in various applications, including food processing and pharmaceuticals.

The Herschel-Bulkley and Power-Law models are employed to describe the relationship between shear stress and shear rate in these solutions. The Herschel-Bulkley model is a generalized model that describes the flow behavior of fluids that exhibit yield stress. Yield stress is the minimum stress required to make a material begin to flow or change shape. The model is given by the equation $\tau = \tau_0 + k * \dot{\gamma}^n$, where τ represents the shear stress, τ_0 is the yield stress, k is the consistency coefficient, $\dot{\gamma}$ is the shear rate, and n is the flow behavior index. This model is particularly useful for materials that exhibit plastic behavior, meaning they have a yield stress below which they behave like a solid, and above which they flow like a fluid.

On the other hand, the Power-Law model is a simplified version of the Herschel-Bulkley model, which disregards the yield stress term. It is given by the equation $\tau = k * \dot{\gamma}^n$. This model is applicable to fluids that do not have a yield stress, or in cases where the yield stress is negligible compared to the viscous stresses. It is often used to describe the flow behavior of non-Newtonian fluids, which do not have a constant viscosity and whose flow behavior changes with the applied shear rate.

The data from study shows that the flow behavior index (n) for β -glucan solutions ranges from 0.99751 to 1.0251. This indicates that the β -glucan solutions behave like a Newtonian

fluid ($n=1$) at certain concentrations. Newtonian fluids have a constant viscosity, meaning their resistance to flow does not change with the applied shear rate. This behavior is characteristic of many common fluids, such as water and most gases.

However, the yield stress and consistency coefficient of the β -glucan solutions increase with the concentration of β -glucan. This indicates that higher pressures are required to deform the solutions at higher concentrations. This behavior is characteristic of many polymer solutions and suspensions, where the interactions between the particles or molecules increase with concentration, leading to an increase in yield stress and consistency.

The Power-Law model is found to provide the best fit for the flow behavior properties of β -glucan at various concentrations. This suggests that the β -glucan solutions behave as non-Newtonian fluids, with a viscosity that varies with the shear rate. This behavior is common in many polymer solutions and biological fluids, where the interactions between the molecules can change with the applied shear rate.

In conclusion, the analysis of the equations and data in study provides a comprehensive understanding of the rheological properties of β -glucan solutions. The Herschel-Bulkley and Power-Law models provide valuable tools for describing and predicting the flow behavior of these solutions under different conditions. This understanding is crucial for optimizing the use of β -glucan solutions in various applications, from food processing to pharmaceuticals.

New Mathematical Model:

The mathematical modeling of rheological behavior in materials has been discussed in many studies [243]. It presents several equations and models that describe this behavior. Here are the key equations:

Constitutive Equation: This equation describes the relationship between stress and strain in a material. It is given by:

$$\sigma = E * \varepsilon$$

where:

σ is the stress

E is the modulus of elasticity (a material property)

ε is the strain

Newtonian Fluid Model: This model describes the behavior of fluids that have a linear relationship between shear stress and shear rate. The equation is:

$$\tau = \eta * \dot{\gamma}$$

where:

τ is the shear stress

η is the viscosity (a material property)

$\dot{\gamma}$ is the shear rate

Maxwell Model: This model describes the behavior of viscoelastic materials, which exhibit both elastic and viscous behavior. The model is represented by a spring (elastic element) and a dashpot (viscous element) in series. The equation is:

$$\sigma + \lambda * (d\sigma/dt) = E * \varepsilon$$

where:

λ is the relaxation time (a material property)

$d\sigma/dt$ is the rate of change of stress with time

Kelvin-Voigt Model: This model also describes viscoelastic behavior, but it represents the material as a spring and dashpot in parallel. The equation is:

$$\sigma = E * \varepsilon + \eta * (d\varepsilon/dt)$$

where:

$d\varepsilon/dt$ is the rate of change of strain with time

Now, let's create a new mathematical model that combines these concepts. We'll assume a material that exhibits both Newtonian fluid behavior and viscoelastic behavior as described

by the Maxwell model. This could represent a material that behaves as a fluid under certain conditions and as a viscoelastic solid under others.

Our new model could be represented by the following equation:

$$\sigma + \lambda * (d\sigma/dt) = E * \varepsilon + \eta * \dot{\gamma}$$

This equation represents a material that has both a rate-dependent viscosity ($\eta * \dot{\gamma}$ term) and a time-dependent stress relaxation ($\lambda * (d\sigma/dt)$ term). The balance of these two terms would depend on the specific properties of the material (E, η, λ) and the conditions of deformation ($\varepsilon, \dot{\gamma}$).

The rheological properties of β -glucan solutions, as presented in study, exhibit a complex interplay between Newtonian fluid behavior and viscoelastic behavior. This complexity can be captured using a more comprehensive mathematical model, such as the one represented by the equation $\sigma + \lambda * (d\sigma/dt) = E * \varepsilon + \eta * \dot{\gamma}$. This equation represents a material that exhibits both Newtonian fluid behavior ($E * \varepsilon + \eta * \dot{\gamma}$) and viscoelastic behavior ($\sigma + \lambda * (d\sigma/dt)$).

The equation: $\sigma + \lambda * (d\sigma/dt) = E * \varepsilon + \eta * \dot{\gamma}$

In this model, σ is the shear stress, λ is the relaxation time, $d\sigma/dt$ is the rate of change of stress with time, E is the modulus of elasticity, ε is the strain, η is the viscosity, and $\dot{\gamma}$ is the shear rate. The left-hand side of the equation represents the viscoelastic behavior of the material, with the σ term representing the elastic response and the $\lambda * (d\sigma/dt)$ term representing the viscous response. The right-hand side of the equation represents the Newtonian fluid behavior, with the $E * \varepsilon$ term representing the elastic response and the $\eta * \dot{\gamma}$ term representing the viscous response. The viscoelastic behavior of β -glucan solutions is characterized by their ability to behave both as a solid and a fluid. When at rest or under low shear rates, these solutions behave like a solid, exhibiting a certain amount of resistance to deformation. This is represented by the σ term in the equation. However, when subjected to a shear stress, they start to flow like a fluid, with the rate of deformation depending on the applied stress and the viscosity of the solution. This is represented by the $\lambda * (d\sigma/dt)$ term.

On the other hand, the Newtonian fluid behavior of β -glucan solutions is characterized by their constant viscosity, regardless of the applied shear rate. This is represented by the $\eta * \dot{\gamma}$

term in the equation. The elastic response of the material, represented by the $E * \varepsilon$ term, is typically negligible for Newtonian fluids but can become significant for viscoelastic materials. This mathematical model provides a more comprehensive description of the rheological behavior of β -glucan solutions than the Herschel-Bulkley or Power-Law models alone. By adjusting the parameters E , η , and λ based on experimental data, the model can accurately describe the material's behavior under different conditions. For instance, the model can predict how the viscosity of the solution changes with the shear rate, which is crucial in processes such as mixing or pumping. It can also predict how the material responds to a sudden change in stress, which is important in processes such as extrusion or injection molding.

Furthermore, the model can be used to study the effect of concentration on the rheological properties of β -glucan solutions. By fitting the model to experimental data at different concentrations, one can determine how the parameters E , η , and λ change with concentration. This can provide insights into the interactions between the β -glucan molecules and how they affect the material's behavior.

In conclusion, the equation $\sigma + \lambda * (d\sigma/dt) = E * \varepsilon + \eta * \dot{\gamma}$ provides a powerful tool for studying the rheological properties of β -glucan solutions.

4. CONCLUSION

In this study, (1-3)(1-6) β -glucan was obtained from oyster mushroom by KOH alkaline extraction, characterized, and applied to β -glucan model food.

The ideal extraction process parameters were found as 80°C, 90 minutes, and 30% KOH. ^1H and ^{13}C NMR spectroscopy, were used for chemical characterization and extracted β -glucan was found to be a β -(1-3) glucose polymer with β -(1-6) side chains. These results agree with those from FTIR spectroscopy. The molecular weight (M_w) was determined as 349.260 g/mol. β -glucan extract did not present any antimicrobial activity. *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 8739, or *Enterococcus hirae* ATCC 10541. Rheological measurements showed that β -glucan has low apparent viscosity in aqueous solutions.

Lastly, β -glucan was added to the cracker recipe, and the finished product's sensory and physical characteristics as well as its estimated glycemic index were assessed. In 30 g of β -glucan, the lowest HI (30.2%) and eG1 (56.38%) values were found. During the sensory analyses, participants reported that 20% of the β -glucan samples had a slight bitter taste and if this taste was improved, the crackers could be consumed. During the sensory analyses, participants reported that 20% of the β -glucan samples had a slight bitter taste and if this taste was improved, the crackers could be consumed.

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