EXTRACTION OF β-GLUCAN FROM OATS (*Avena sativa*): A COMPARATIVE STUDY

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Roll No. 0119/09

Registration No. 00667

Session: January-June, 2019

A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Applied Human Nutrition and Dietetics

Department of Applied Food Science and Nutrition

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JUNE 2021

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June, 2021

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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JUNE 2021

DEDICATATION

I Dedicated My Small Piece of Work

to My Beloved Family Members and Respected Teachers.

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List of Abbreviations

β-G	Beta Glucan
LDL	Low Density Lipoprotein
HDL	High Density Lipoprotein
rpm	Revolutions per minute
SDF	Soluble Dietary Fiber
LDLR	Low Density Lipoprotein Receptor
MW	Molecular Weight
RT	Room Temperature
GI	Glycemic Index
BMI	Body Mass Index
PI3K	Phosphatidylinositol-3-Kinase
Akt	Protein Kinase B
GLUT4	Glucose Transporter Type 4
Ppt	Precipitation
EtOH	Ethanol
PPAR-γ	Peroxisome Proliferator Activated Receptors-γ

Abstracts

With snowballing interest in eating healthily in the present century, as well as the endemic prevalence of obesity, great focus is being placed on providing consumers solubilized fibers through food. Cereals compensate 73% of all farmed land worldwide, making them clear and significant sources of beneficial insoluble and soluble fiber. Oat (*Avena sativa*) β-glucan is a soluble dietary fiber, useful functional component with a variety of industrial, nutritional, and health advantages. Oats are claimed to stand out among cereals because they are more efficacious than other grains, like wheat or rice, which are primarily insoluble, against conditions including hyperglycemia, dyslipidemia, high blood pressure, inflammation, and vasculitis. There are a plethora of sources for this vital component. β-Glucan can be derived from various sources to meet the ever-increasing need for nutraceutical products. The focus of this research was to assess the efficiency of several extraction processes in terms of β-glucan yield and recovery. The extraction of β-glucan from oats was studied using three different methods. The highest yield (6.94%) and recovery were obtained in alkaline-extracted oat samples followed by 4.54% when hot water extraction procedure was used, whereas lowest yield (3.70%) was obtained in acidic extraction. Alkaline and acidic approach yielded the most soluble fiber, while the hot water extracted sample yielded the least amount of insoluble fiber (0.76%). The presence of β-glucan in extracted beta glucan gum pellets is also confirmed by UV spectroscopy. The percentage of glucose concentration in extracted β-glucan was highest in alkaline yield (0.38%) followed by 0.22% in hot water yield, whereas lowest 0.07% was observed in acidic yield. Overall, isolated beta glucan showed great promise for commercial applications. The extraction method may change the physiochemical and functional aspects of extracted β-glucan, thus it must be done with caution.

Keywords: β-glucan, oats, dietary fiber, extraction methods

Chapter-1: Introduction

As obesity, diabetes, and heart disease become more prominent, the value of a high-fiber diet is becoming more clear. Oat (*Avena sativa*) has been known as an excellent source of soluble dietary fiber for more than two decades. Mixed linkage β-glucans ((1-3) (1-4) β-D-glucan) are a type of linear poly-glucose fiber found in oats that has important nutritional features (American Association of Cereal Chemists International, 2001). Cereal grains are a staple food in nearly every country on the planet. These grains are a good source of dietary fiber, accounting for nearly half of all fiber consumed in Western countries. Barley and oats are the cereals with the most soluble fiber components, particularly β-glucan. Oat (*Avena sativa*) is still a popular cereal crop in developing countries, and it's utilized for both human and cattle feed. The usage of oats grain as an animal feed has been steadily declining, which could be linked to a growing interest in oats as a human health food (Tiwari and Cummins, 2009).

Chemically Beta glucan is a non-starch polysaccharide made up of repeated glucose units that can be branched in a variety of ways depending on the source. Long chains of β-D-glucopyranose are joined together via β-(1-3) linkage to form a long backbone, whereas side chains are formed via β-(1-4) linkage, according to the chemical structure. The majority of the β-glucan molecule's structure is made up of β-(1-3) linked cellotriosyl and cellotetraosyl units. The β-1, 3 linkages breakdown the β-D-glucan molecule's regular shape, making it hydrophilic and flexible, therefore it constitutes a significant portion of the water extractable fiber percentage (Manthey et al. 1999). The molecular weight, solubility, and concentration of glucan all affect its ability to generate viscous solutions because of the presence of (1-4) and (1-3) linkages (El et al., 2012; Wood et al., 2000). At low and high shear rates, glucan aqueous dispersions exhibit Newtonian region and shear-thinning flow, accordingly (Lazaridou and Biliaderis, 2007). Cereals, mushrooms, yeast, algae, and beans are just a few of the many sources for this useful component that have been identified. The majority of the cholesterol lowering effects are provided by β-glucan derived from either of the sources. In nutritional supplements, however, β-glucan derived from mushrooms and baker's yeast acts as an immune system enhancer. (Woodward et al., 1983; Wood et al., 1994). These water-soluble sulfated curdlan-enucleotide complexes may have uses in gene technology and medicine, according to the researchers (Zhu et al., 2016). The use of a flexible hydroxyapatite/glucan composite as a bone substitute has been demonstrated

(Belcarz et al., 2013). β-glucan was employed as a new vaccination platform and antigens and immunomodulators can be loaded into β-glucan, which is then released after phagocytosis (Levitz, 2014). White rice combined with high-glucan barley could help prevent and treat obesity and other metabolic problems linked to obesity (Aoe et al., 2014). It offers a variety of useful properties for human health, including the ability to reduce colorectal cancer risk, increase stool volume, and relieve constipation (Ishurd and Kennedy, 2005). Furthermore, higher intestinal viscosity and increased facial mass as a result of β-glucan ingestion can prevent several serious ailments as well as reduce hunger, which could be employed to lose weight (Monro, 2002). Diabetic individuals may benefit from a diet high in β-glucan because it lowers postprandial hyperglycemia and insulin secretion (Lazaridou and Biliaderis, 2007). Prebiotic characteristics of β-glucan have been discovered, and it can specifically increase the activity and proliferation of probiotic bacteria such as lactobacilli and bifidobacteria (Jaskari et al., 1998). A daily consumption of 0.75 g of β-glucan has been shown to have a considerable bifidogenic impact (Mitsou et al., 2010). Cereal β-glucans can be used to enhance the nutritional value and diversity of functional foods in a wide range of food compositions. As a soluble dietary fiber, β-glucan offers a variety of biological functions and health advantages. It also contains physical features such as gelling capacity, stabilizer, emulsifying, and thickening agent that make it suited for use in the food, pharmaceutical, and cosmetics sectors. It's a prebiotic, a texturizer, and a fat substitute in dairy products (Khorshidian et al., 2018). As a result, consumer demand for this polysaccharide is fast increasing, and new culinary products are sprouting (Ahmad et al., 2010). In addition to β-glucan, the cell wall of oats contains protein matrix, lipids, carbohydrates, and minerals. As a result, the purity, yield, structure, physicochemical, functional qualities, and integrality of the β-glucan are all influenced by the extraction, purification processes, and circumstances used (Ahmad et al., 2010; Ahmad et al., 2009).

The extraction procedure could have an impact on the molecular structure. The structure of β-glucan can be considerably affected by enzymes such as grain's endogenous β-glucanases in the aqueous environment, as well as shear-induced molecular fragmentation that happens during mixing and centrifugation processes. The soluble β-glucan, in particular, is more impacted by such degradation, which results in a drop in molecular weight and viscosity, which reduces its cholesterol-lowering and glucose-

attenuating effects (Bhatty, 1993). Barley gum's yield, composition, and viscosity were all evaluated with respect to the extraction conditions. The functional behavior, composition, viscosity, color, molecular weight, and physicochemical properties of the extracted β-glucan may all be strongly influenced by a little change in the extraction process (Burkus and Temelli, 1998). Endogenous enzyme inactivation, β-glucan extraction, and β-glucan precipitation are the three processes involved in its extraction from cereals (Irakli et al., 2004). Previously, scientists devised a variety of extraction procedures for use in the laboratory. Hot water extraction, enzymatic extraction, solvent (acidic) extraction, and alkaline extraction are the four methods available nowadays (Ahmad et al., 2009). A study indicated that enzymatic extraction yields a better yield than acidic or alkaline extraction, yet protein was found to be a prominent contaminant in this study.

There is no question today that glucan can provide a variety of nutritional and rheological benefits to food products, and the food industry always exhibits a keen interest in the physiochemical and functional properties of novel nutraceutical compounds as this will aid in the selection of a specific type of compound with a particular set of characteristics for a specific food product (Ahmad et al., 2010). The physiochemical and functional qualities of extracted glucan may be impacted by the extraction conditions. This study was therefore designed with the objective of comparing the effectiveness of extraction techniques for yield and recovery on significant physiochemical parameters of β-glucan having key industrial uses.

Aims & Objectives

- To extract soluble dietary fiber (β-glucan) from oats (*Avena sativa*) in different extraction procedures.
- To quantify the β-glucan through spectrophotometric analysis & total fiber content determination.
- To compare efficiency of different extraction procedures in terms of β-glucan yield percentage.

Chapter-2: Review of literature

The β-Glucan (a soluble dietary fiber) has a wide range of biological function, for example, it is effective against a variety of chronic diseases such as diabetes, cancer, and improper digestion. β-Glucan is a D-glucose polysaccharide. It can be extracted from a variety of sources, including grains, yeasts, fungi, and bacteria. As β-Glucan is a natural substance that can be employed in pharmaceutical products for the prevention and treatment of many chronic diseases, its extraction has become extremely essential in recent years. Many food producers, including as dairy, meat, and bakery items, are also interested in incorporating the β-Glucan into their products (Ibrahim and Selezneva, 2017).

Because of their health benefits, oats have been designated as a "Supergrain" Oats and oat products contain a variety of bioactive substances that may have health advantages in addition to soluble fiber, polyphenols, and avenanthramides (antioxidants). Oats and oat-containing products that have a certain amount of oat beta-glucan can carry an FDA-approved health claim about cholesterol-lowering benefits (Khanna and Mohan, 2016).

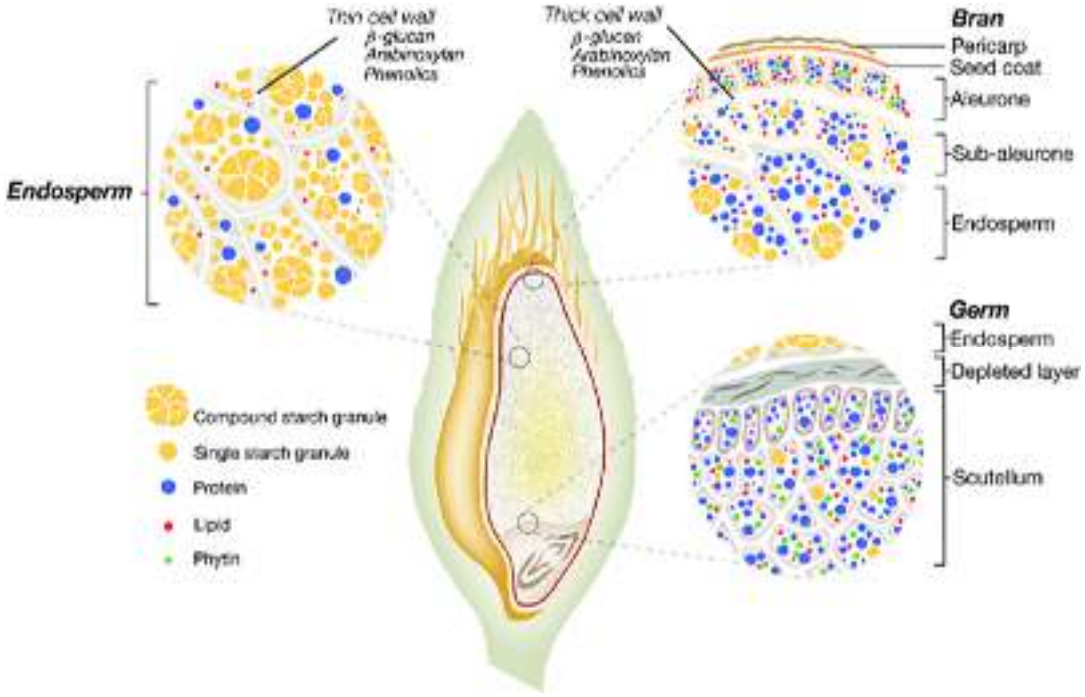

Figure 2.1 Structural representation and nutrient distribution of the oat grain presenting different oat tissues (Grundy et al., 2018).

2.1 Oats (*Avena sativa*)

Oats are one of the foodstuffs that are most abundant in dietary fiber among cereals, like all other grains, is a member of the Poaceae family and is known in India as "Jai" or "Javi." The common oat (*Avena sativa*) is a cereal grain that is primarily farmed for use as oatmeal and livestock feed. Without a good understanding of its precise health related benefits, oat has always been viewed as a health-promoting food. However, it is currently renowned for its effects on satiety and delayed food absorption, as well as a deterrent to a variety of gastrointestinal problems. The soluble fiber content of oats is primarily responsible for these positive effects (Khanna and Mohan, 2016).

Table 2.1 Classification of oats (*Avena sativa*)

Kingdom	***Plantae* - Plants**
Subkingdom	*Tracheobionta* - Vascular plants
Superdivision	*Spermatophyta* - Seed plants
Division	*Magnoliophyta* - Flowering plants
Class	*Liliopsida* - Monocotyledons
Subclass	*Commelinidae*
Order	*Cyperales*
Family	*Poaceae / Gramineae* - Grass family
Genus	*Avena* L. - oat
Species	*Avena sativa* L. - common oat

Source: USDA plants database (2005)

Oats have been grown in various parts of the world for over two thousand years. Oats are farmed all over the world in temperate climates. They require less heat in the summer and are more tolerant of rain than other cereals such as wheat, rye, or barley. They're especially helpful in places where the summers are chilly and damp. Oats are an annual plant that can be planted in the fall (harvested in late summer) or the spring (harvested in early autumn) (Dimberg et al., 1996). Oats are categorized into numerous categories depending on their degree of processing. For human consumption, the hard outer hull of whole oats must be removed. "Groats" refers to oats that have been hulled. Furfural is a chemical that can be found in oat hulls. Steel-cut oats, commonly known as pinhead oats, are prepared by passing groats through steel cutters that chop each one into three or four pieces. They're high in nutrients because they're made with whole

grains, including oat bran. The groats are steamed and then flattened with a roller to make rolled oats. Instant oats are manufactured in the same way as rolled quick-cooking oats, with the exception that they are steamed for a longer time and rolled thinner. Ground oats produce oat flour, which comes in three grades: coarse, medium, and fine (Winfield et al., 2007).

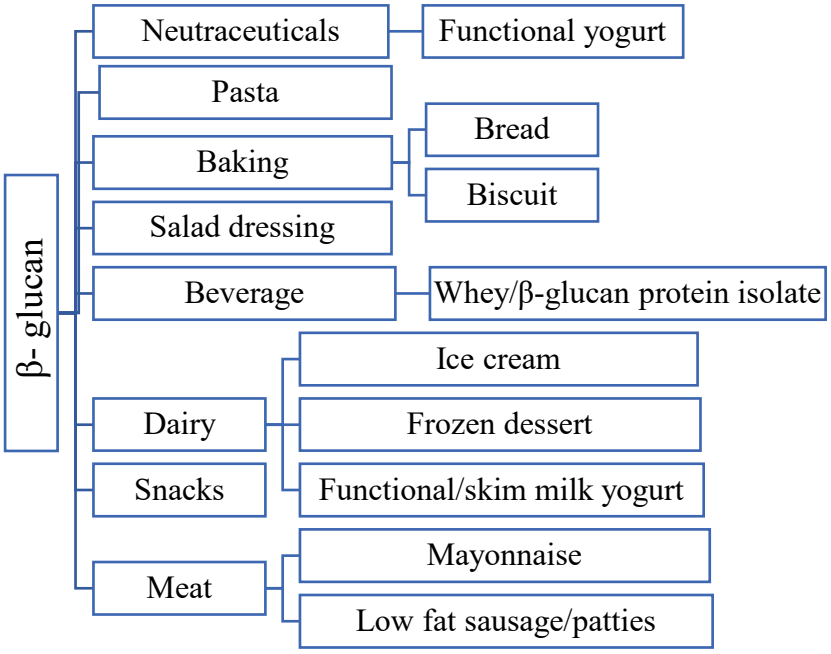

Figure 2.2 Prospective of β-glucan for various food products (Ahmad and Ahmed, 2016)

Food processors can use -glucan and other dietary fibers to manage synersis, gelling, emulsification, and water-holding capacity, as well as improve/modify oil holding ability. Bakery products, jams, jellies, soups, meat products, and dairy products all require these processes (Ahmad et al., 2012). Cereal -glucans have sparked a lot of interest because of their potential as a beneficial dietary fiber (Du et al., 2014). The wild red oat, a plant native to Asia, is thought to be the predecessor of modern oat. Oats have been grown in various parts of the world for over two thousand years. The use of oat as a food and for therapeutic purposes has been promoted (Singh et al., 2013).

2.2 Nutritional profile of oats (*Avena sativa*)

Oats are often regarded as "healthy," with commercial marketing touting them as "nutritious," leading to a greater acceptance of oats as a human food. All three of the grain's components, the germ, endosperm, and bran, which are all rich in nutrients, are found in oat grout or whole grain (after the hull is removed) (Figure 2.2). In addition to having a high quantity of antioxidants, vitamins, and minerals, oats are also high in total protein, carbohydrates, crude fat, and dietary fiber (Table 2.2) when compared to other cereals (Sangwan et al., 2014). Dry matter can include up to 75% to 80% of its weight in total carbohydrates, including cellulose and non-starch polysaccharides. Starch is the most important component of oats, and its amount varies according on the type and growth conditions. The amylase concentration of oat starch varies between 16 and 18 percent and 28.5 and 28.7% (Mirmoghtadaie et al. 2009).

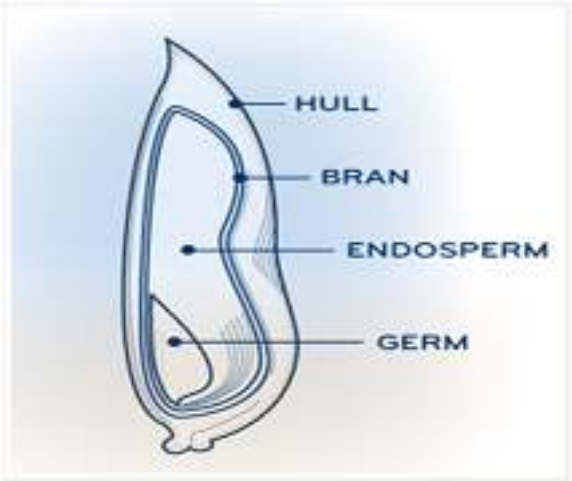

Figure 2.3 Structure of oat grain (quakeroats.com)

Oats are nutritionally superior to other cereals due to their high oat protein content and well-balanced amino acid profile. (Brand and Merwe, 1996; Petkov et al., 2001). Only oats are a cereal whose majority of grain protein is classified as globulins because it is salt soluble, with very little albumin and prolamin content that is alcohol soluble. Oats and their byproducts include phenol compounds with a strong antioxidant activity. (Sobotka et al., 2012).

The most active oat proteins in terms of metabolism are the enzymes. Several enzymes are found in oat grout, as they are in other cereal grains. Proteases, maltase, α-amylase, lichenase, phenoxyacetylase and hydroxylase, phosphatase, tyrosinase, and lipase were discovered in the early stages of the inquiry (Caldwell and Pomeranz, 1973).

Triglycerides make up the majority of lipids, but phospholipids, glycolipids, and sterols are also found in significant amounts. Starch retrogradation in oats is slowed down both in terms of rate and amount due to the high lipid content of oat starch (Gudmunsson and Eliasson, 1989).

Roughage or bulk, also referred to as dietary fibers (non-starch polysaccharides), are edible plant parts that are essential for human nutrition. Despite the fact that they are not a nutrient, they are a vital part of our meals. After passing through the small intestine, dietary fiber is partially or completely fermented in the large intestine by gut bacteria. The fermentation process results in the production of numerous by-products, including gases and short chain fatty acids. The combined impact of the fermentation process and byproducts produced is thought to be the cause of dietary fiber's beneficial benefits on health. Oats have a well-balanced dietary fiber profile, both soluble and insoluble. A high dietary fiber intake has been linked to a number of medicinal and nutritional benefits. For instance, dietary fiber compound, which contains antioxidant properties and other phytochemicals, is extremely beneficial for lowering cholesterol and avoiding cancer and cardiovascular disease (Jacobs et al., 1998a; Jacobs et al., 1998; Slavin et al., 2000; Thompson, 1994).

Table 2.2: Nutritional profile of whole grain oats and oat brans

Nutrients	Whole grain oat	Oat bran
Protein	15-17%	15-18%
Starch & Sugar	59-70%	10-50%
Fat	~ 4.5%	~ 6.5%
Total Dietary Fiber	~ 12%	~ 14-15%
β - Glucan	2-6%	5-20%
cellulose	14%	~ 2.5%
Lignin	~ 2.5%	~ 4.5%

Source: (Usman et al., 2010)

Phosphorus, potassium, magnesium, and calcium are the key components of oat's mineral content (Table 2.3), which is 2-3%. The water-soluble vitamin content of oats

is outlined in (Table 2.4). Oats have a folate level of 20-30 g/100g grain and a biotin concentration of 10-15g/100g grain.

Table 2.3: Mineral composition of oats (USDA, 2005)

Mineral content in oats (1 cup/156g)	
Calcium, Ca	84 mg
Iron, Fe	7.36 mg
Magnesium, Mg	276 mg
Phosphorous, P	816 mg
Potassium, K	669 mg
Sodium, Na	3 mg
Zinc, Zn	6.19 mg
Copper, Cu	0.977 mg
Manganese, Mn	7.669 mg

Table 2.4: Vitamin composition of oats (USDA, 2005)

Vitamin content in oats (1 cup/156 g)	
Vitamin C, total ascorbic acid	0.0 mg
Thiamin	1.190 mg
Riboflavin	0.217 mg
Niacin	1.499 mg
Pantothenic acid	2.104 mg
Vitamin B_6	0.186 mg
Folate, total	87 µg
Folic acid	0 µg
Folate, food	87 µg
Folate, DFE	87 µg
Vitamin B_{12}	0 µg
Vitamin A, IU	0 µg
Retinol	0 µg

Oats' antioxidants are essential for the stability of oat products during storage because they stop lipids from oxidizing. Oats include a lot of tocopherols, which are antioxidants and have an average concentration of 2.3 mg per 100 g of grain (Morrison et al., 1978; Peterson, 1995). Furthermore, oat grains contain a large number of low molecular weight phenolic chemicals. Natural plant antioxidants play an essential role in the prevention of several diseases, according to recent medical and nutritional research. Our understanding of phenol chemicals in oats has grown thanks to new study. Avenanthramides are a type of phenolic antioxidant found in oats. Cinnamoyl-anthranilic acid derivatives are what these chemicals are (Figure 2.5) depicts the structure of two avenanthramides (Collins, 1986; Dimberg et al., 2005)

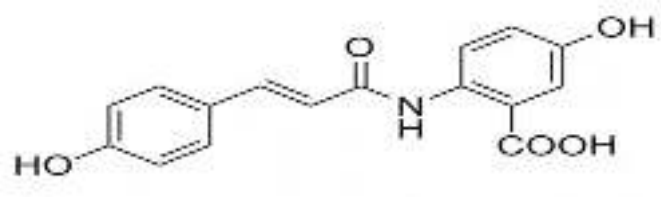

Figure 2.4 Structure of Avenanthramides (Dimberg et al., 2005)

Table 2.5: Dietary fiber and β-glucan content in different oat products

Fiber (%)	Oat endosperm flour	Whole grain products	Conventional oat bran products	Oat bran concentrates	β – glucan isolates
Dietary fiber	5-10	10-12	15-20	20-35	80-100
β - glucan	1-3	4-5	8-12	15-22	Up to 80

Source: (Manthey et al., 1999)

2.3 Chemical composition of oat β-glucan (a biological defense modifier)

The soluble dietary fiber called β-glucan, which is also a mixed-linkage polysaccharide containing D-glucose units, nutritionally potentiates and modulates the immune response. Although the bonds between D-glucopyranosyl units in β-glucans are primarily composed of β-1,3 (cellotriosyl units) and β-1,4 (cellotetraosyl units) linkages, there are some portions that resemble cellulose and have four or more consecutive β-1,4 linked glucose units (Wood et al., 1993). The β-1, 3 linkages break

up the β-D-glucan molecule's regular structure, making it liquid and flexible, for that it is an important element of the water recoverable fiber fraction. (Manthey et al., 1999).

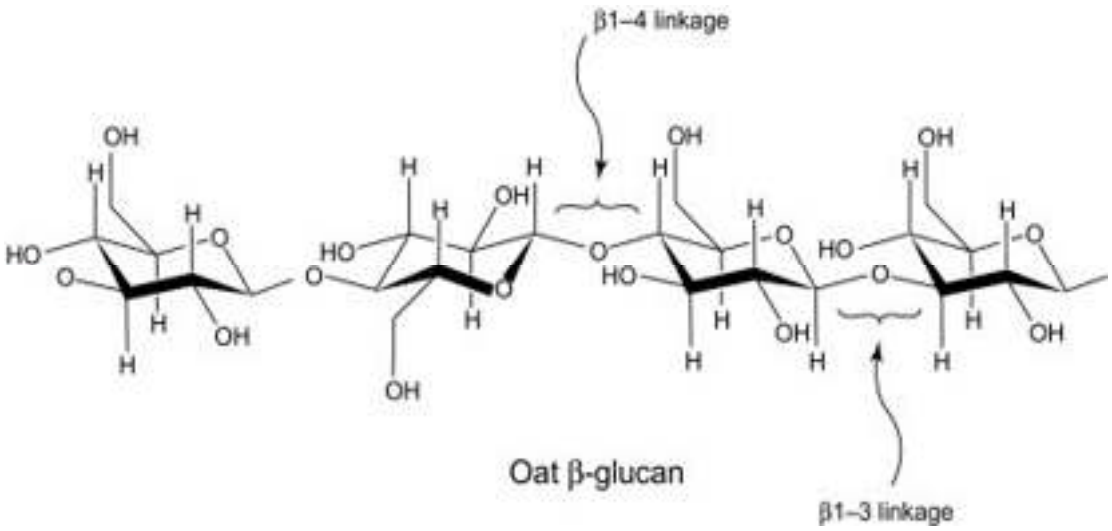

Figure 2.5 Linkage of β – glucan (Menon et al., 2016)

2.3.1 β–glucan's position in oats

In the dry weight of the whole grain oats, β-glucan makes up 3.6–5.1%. (Hampshire, 2004). Variation in cultivars and environmental factors that affect growth, such as nitrogen content, temperature, and rainfall, all have an impact on β-glucan concentration (Asp et al., 1992; Brunner and Freed, 1994). β-glucans can be found all over the starchy endosperm. They're mostly found in the bran, specifically the aleurone and sub-aleurone layers (Fig. 2.6).

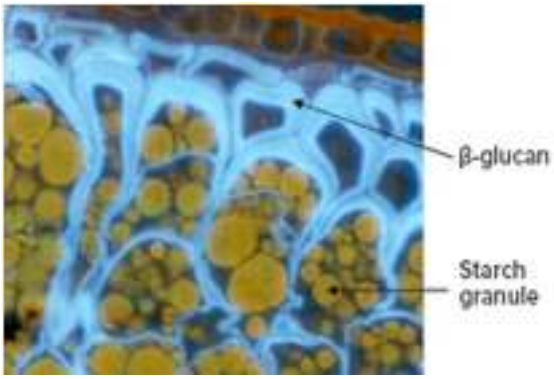

Figure 2.6 position of β-glucan in oat grain (oatsandhealth.org)

2.4 Therapeutic attributes of oats against diabetes & others metabolic disorders

Oats stand out among cereals because of their multifunctional properties and unique nutritional profile. Diabetes and cardiovascular problems have been shown to benefit from oats and oat products (Khanna and Mohan, 2016).

2.4.1 Oats against dyslipidemia

Oats containing β-glucan soluble fiber, which aids in cholesterol reduction. 3 grams of soluble fiber per day from oats, together with a low-saturated-fat, low-cholesterol diet and a healthy, active lifestyle, can help lower cholesterol (USFDA guidelines).

The viscosity of β-glucan is connected to its strong transport of bile acids towards lower regions of the intestinal tract and high excretion of bile acids, decreasing serum cholesterol levels and attenuating postprandial plasma glucose levels. Cholesterol levels that are too high are a key risk factor for cardiovascular disease. Increasing dietary fiber has been suggested as a safe and feasible method for lowering cholesterol (as a helpful supplement to lipid-lowering diets). The ability of oats to lessen the risk of heart disease has been demonstrated (USFDA, 1997).

- The hypocholesterolemic impact of oat fibre may be due to the interplay of four different pathways. First, oat fiber affects bile acid metabolism and increases fecal bile excretion. Second, oat bran may affect lipoprotein metabolism by enhancing hepatic LDL receptors, which could lead to changes in lipoprotein metabolism. Oats have a stronger tendency to reduce LDL cholesterol while raising HDL cholesterol. Third, after being absorbed into the portal vein, oat bran is fermented in the colon to create short-chain fatty acids like acetate, propionate, and butyrate, which may reduce the production of hepatic cholesterol. Fourth, lower insulin secretion linked to fibers like oat bran may result in lower cholesterol production.
- Oat fiber favorably modulates atherogenic fasting and postprandial blood lipids and other lipoprotein fractions irrespective of other dietary alterations, so it has been demonstrated to be helpful in lowering the risk of CHD (Butt et al. 2008). As a result, it is stressed that consuming oats and oat products is crucial in lowering the risk of cardiovascular disease (Singh et al. 2013).

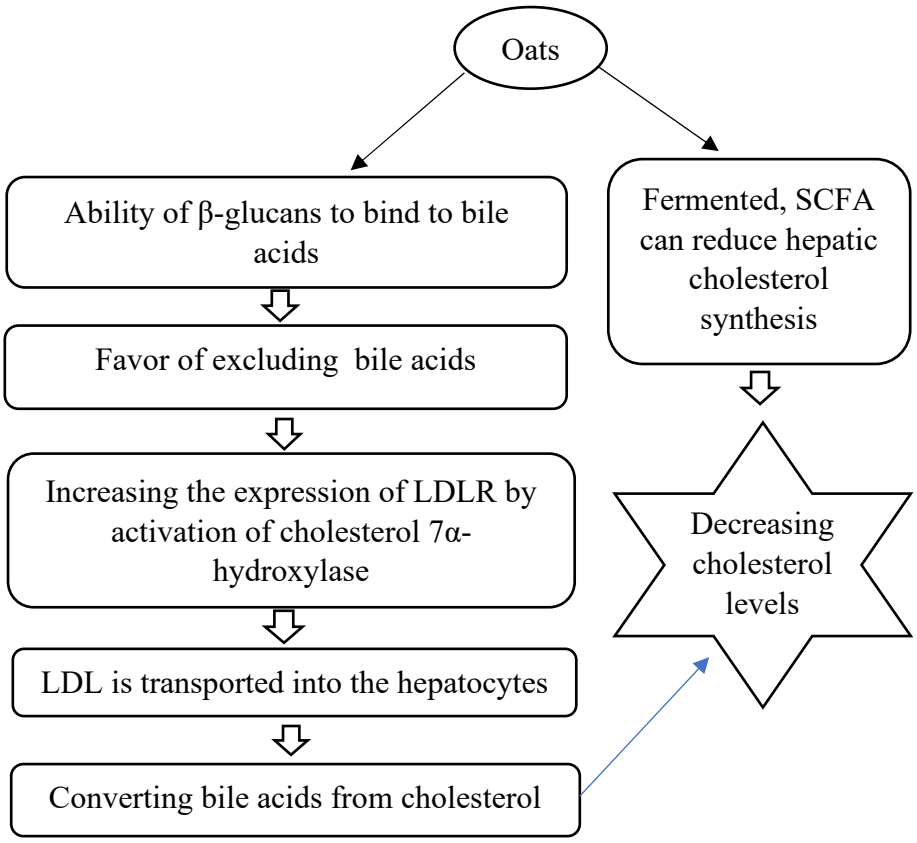

Figure 2.7 Prevention of dyslipidemia (Marlett, 1997; Ellegard and Anderson, 2007)

2.4.2 Oats help to manage weight

Dietary fiber is abundant in oats. Fibre-rich diets may assist with weight maintenance. Fiber, according to epidemiological and clinical evidence, can help with weight management and/or the prevention of weight gain and obesity (body mass index >30%). Fiber may provide a textural characteristic that encourages people to chew for longer periods of time (which leads to satiation). Gastric emptying, small intestinal transit time, and carbohydrate and fat digestion and absorption have all been observed to be slowed by viscous fibers (such as those present in oats). These effects have been demonstrated to increase satiety and aid in energy intake control by successfully altering the glycemic response. Cholecystokinin (CCK), a hormone that promotes fat-induced satiety, is elevated in plasma in response to beta-glucan-enriched foods. Protein is also more satiating than isoenergetic levels of carbohydrate or fat, and oats have the highest protein content of all the main cereals. Oats' amino acid profile (low lysine to arginine ratio) has also been found to be hypocholesterolemic and cardioprotective (Khanna and Mohan, 2016).

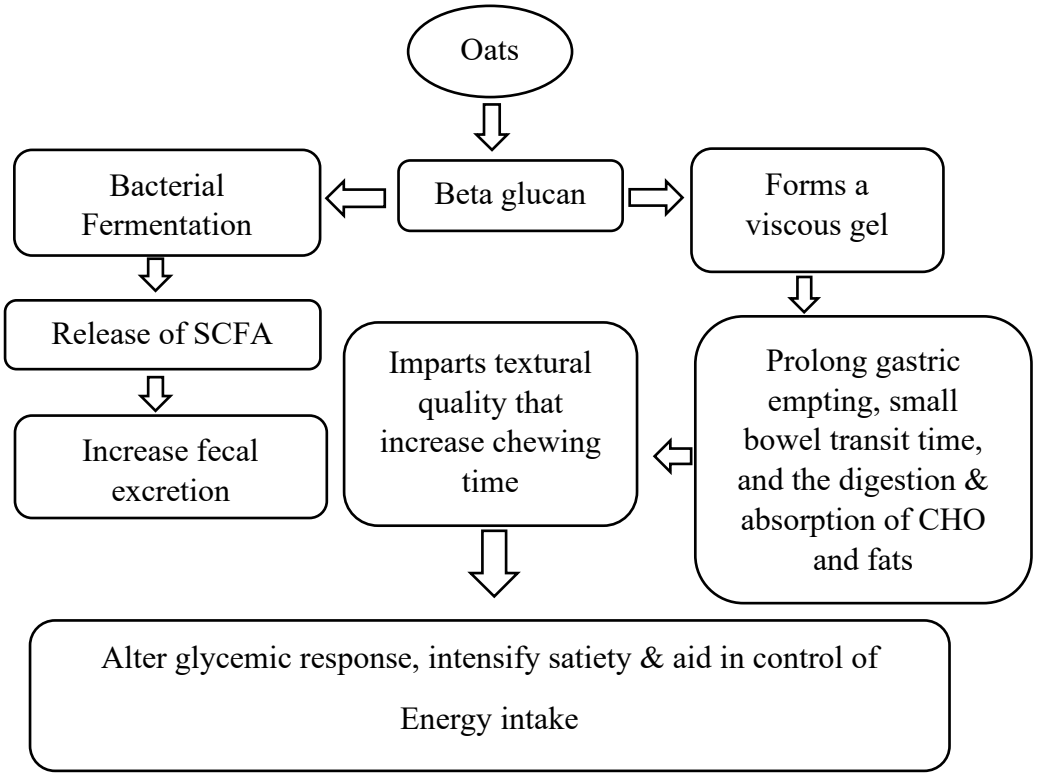

Figure 2.8 Mechanism of action to lose weight (Khanna and Mohan, 2016)

2.4.3 Oats combat type 2 diabetes

Oat beta-glucans have been shown to be effective in treating insulin resistance, which is a key factor in the development of the metabolic syndrome, a condition associated with type 2 diabetes. The findings that a high dietary fiber intake is associated with greater insulin sensitivity are confirmed by the finding that total fiber consumption, as well as soluble and insoluble fiber intake, is adversely related to insulin resistance (Galisteo, 2008; Ylonen et al., 2003). Oat glucans were used in numerous clinical trials to reduce blood sugar levels. Oats provide a multitude of health benefits that lower blood sugar and prevent diabetes. Beta glucans, which are found in oats and have the capacity to gel, help control blood sugar levels (particularly for cases of type II diabetes) (Butt et al., 2008). Consequently, dietary glucose is absorbed more slowly (Kiho et al., 1995).

Studies show that oat β-glucan (5 g daily) reduces the glycemic index by up to 50% after ingestion of 35 g of carbs (Tappy et al., 1996), or each gram of β-glucan lowers the GI by 4 units in a meal containing 50 g of carbohydrates (Jenkins et al., 2002). After eating the oat (bran flour or crisp), blood glucose levels dropped at 15, 30, and 45

minutes. However, they spiked at 90 minutes after taking 12.5g of glucose (Tapola et al., 2005). The feeling of hunger brought on by a sudden drop in blood glucose is lessened by these changes (Ludwig, 2003; Saris, 2003). As a result, β-glucans suppress appetite and eat less. Oatmeal diet days, according to Zerm et al, may help people with type 2 diabetes mellitus reduce their insulin resistance (Zerm et al., 2013).

Oat-glucans can also raise the PI3K/Akt signaling pathway, which in turn lowers blood sugar levels. It has been discovered that type 2 diabetes can develop as a result of decreased PI3K/Akt activity. Numerous receptors have been implicated in the stimulation of PI3K/Akt by glucans (Hsu et al., 2002; Chen and Seviour, 2007). One group of receptors induced by β-glucans is dectin-1, which also stimulates complement receptor 3 lactosylceramide, scavenger, and toll-like receptors. Each of these receptors activates a distinct signal pathway. Brown (2006) and Chen and Seviour (2007) studied how beta-glucans and their receptors interacted. The treatment of β-glucans may be able to restore decreased PI3K in diabetes because mushroom extract has been shown to activate the PI3K pathway (Li et al., 2006; Lee et al., 2008).

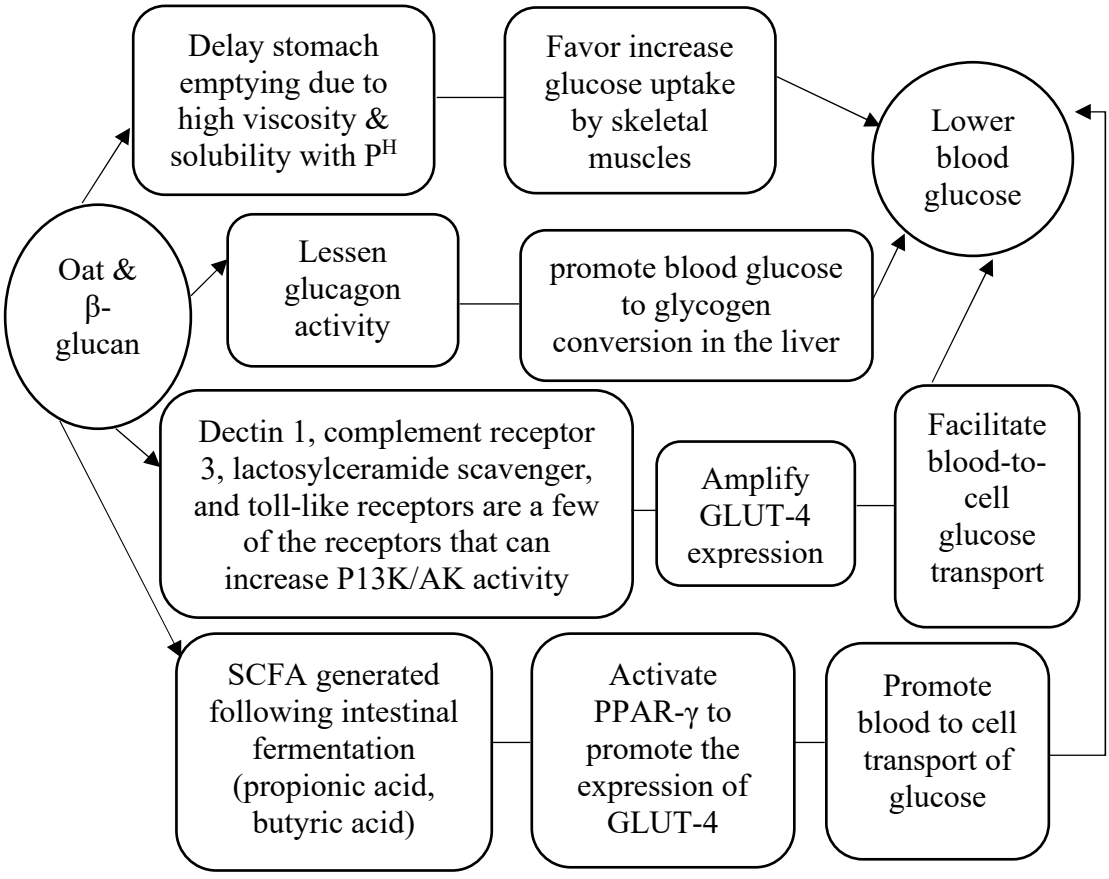

Figure 2.9 Mechanism of oats' anti- diabetic effects (Butt et al., 2008)

2.4.4 Oats may help to reduce hypertension

In a clinical research with diet containing oat-glucans, participants with BMIs above the median (31.5 kg/m2) had lower blood pressure (Maki et al., 2007). Another study found that oats are useful in the treatment of hypertension (He et al., 2004). Several mechanisms have been proposed to explain how dietary fiber intake may affect blood pressure. Dietary fiber has a variety of effects on food digestion and absorption. Significant underlying pathologic pathways for the development of hypertension have been suggested to include insulin resistance and the compensatory hyperinsulinemia that goes along with it (Ferrannini et al.,1987).

2.4.5 Oats against proinflammatory state

Most studies on weight loss have shown that lowering body weight is linked to lowering CRP (C reactive protein) (Dietrich et al., 2005), In obese women, TNF (Tumor Necrosis Factor) (Dandona et al., 1998) and circulating adiponectin levels rise (Esposito et al., 2003). King (2005) asserted in a study that dietary fiber lowers lipid oxidation, which is associated with less inflammation. For fermentable fibers, the production of butyrate may also be responsible for these anti-inflammatory effects. It has been demonstrated that butyrate has anti-inflammatory properties in conditions such inflammatory bowel disease (Patz et al., 1996), and Various human cells types, including macrophages and monocytes, have demonstrated that it has anti-inflammatory effect (Segain et al., 2000).

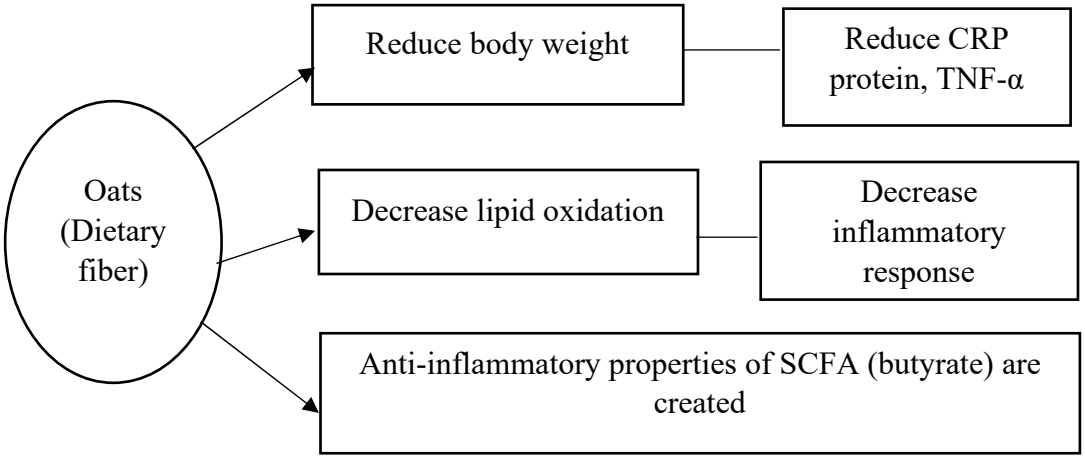

Figure 2.10 Proinflammatory effects of oats (King, 2005)

2.4.6 Oats lessen the risk of cancer

Hong et al. recently advanced the concept that orally administered beta-glucan can act as an adjuvant when combined with exogenously administered antitumor antibodies that activate the components, and demonstrated that this mechanism of action is effective against a wide range of cancers when combined with specific monoclonal antibodies that activate or cause the complement to bind to the tumor. The complement helps these primed neutrophils locate and adhere to the tumor, making it easier for them to kill it. The body's first line of defense is innate immune cells, which circulate throughout the body and engage in an immunological response to "external" assaults (bacteria, fungi, parasites). Neutrophils aren't usually involved in the destruction of malignant tissue since they consider cancer to be "self" rather than "nonself." Monoclonal antibodies and vaccines are now used in cancer immunotherapy, however they only boost the acquired immune response and do nothing to affect the innate immune system's perception of cancer as "self." As a result, monoclonal antibodies alone do not engage or initiate the innate immune system's potential killing power, which is our principal defense against bacteria and yeast (fungal) diseases (Hong et al., 2004). Orally administered β-glucan has recently been discovered to greatly boost monocyte proliferation and activation in the peripheral blood of patients with metastatic breast cancer (Demir et al., 2007).

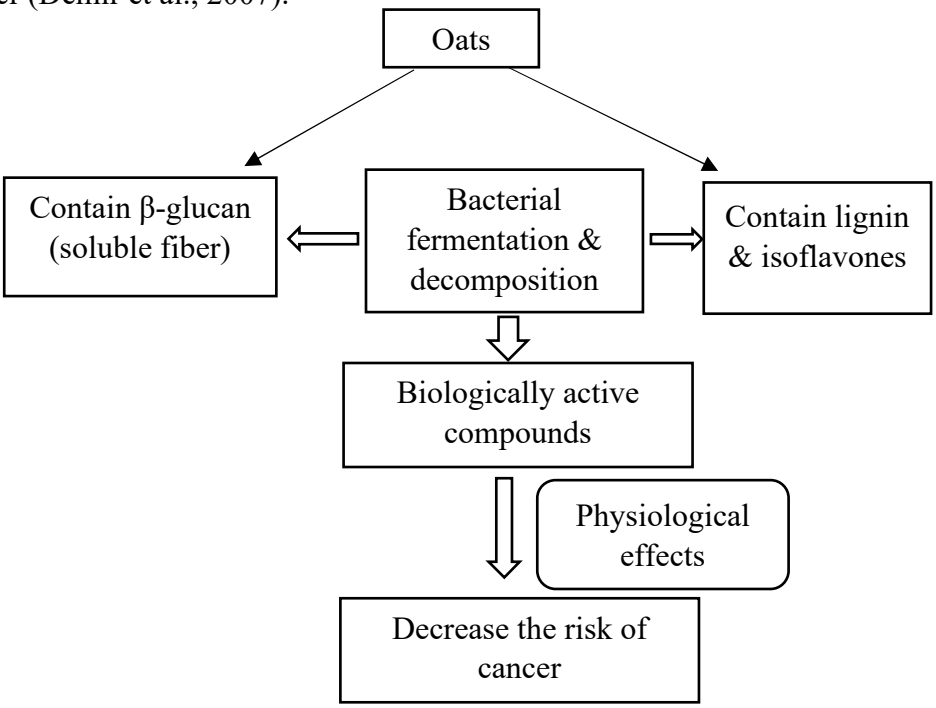

Figure 2.11 Mechanism of action of β-glucan against cancer (Perry and Ying, 2016)

Beta glucan is entirely degraded by bacterial enzymes in the large intestine and acts as a substrate for fermentations in the same way as other soluble fiber sources do. The decrease of the risk of intestinal and other malignancies is undoubtedly one of the most important physiological effects of these fermentations. The presence of lignin and isoflavones (phytoestrogens) in oat fiber provides a distinct advantage. Intestinal bacteria convert phytoestrogens into physiologically active compounds. These can lessen the risk of breast cancer, prostate cancer, and colon cancer over time (Perry and Ying, 2016).

2.5 Extraction procedures of β-glucan

The two most common methods for extracting -glucan, notably from grains, are dry separation and wet separation. The advantage of dry separation is that it does not require the use of a solvent for extraction; nevertheless, the yield from this approach is often less than 30% (Zheng et al., 2000). Wood et al. were the first to adopt the wet method for glucan extraction, and it has since become the preferred method for many food scientists due to its high yield, which typically varies from 30–70 percent (Benito-Román et al., 2011). β-Glucan, like other non-starch polysaccharides, is found in the endosperm cells of barley and oats, which cover the grain's starch, lipid, and protein reserves, making it difficult to recover. The approach to be followed while examining β-glucan physiochemical properties must optimize purity, yield, and maintain the integrity of the β-glucan molecule. The method of extraction has an impact on the β-glucan molecular weight profile as well (Tosh et al., 2003; Wang et al., 2003).

This is because the viscosity, molecular weight, and solubility of β-glucan are primarily responsible for its cholesterol-lowering and glucose-lowering actions (Wood, 2007). Endogenous enzyme inactivation, β-glucan extraction, and β-glucan precipitation are the three processes involved in its extraction from cereals. Endogenous β-glucanases must be inhibited because they can breakdown β-glucan, causing it to lose molecular weight and lose its functional qualities (Irakli et al., 2004). Treatment of barley flour with dilute aqueous ethanol at temperatures over 60°C inactivates these enzymes. Because starch is coextracted at temperatures over 60°C (gelatinization temperature), starch must be eliminated from the extract (Wood et al., 1977). Wood invented a crucial method for extracting β-glucan from oats and

barley. On a laboratory scale, the effects of particle size, pH, ionic strength, and temperature on β-glucan yield were investigated, and on a pilot plant scale, oat-bran enzymes were inactivated with 75 percent ethanol and a sodium carbonate solution at pH 10 to create a 78 percent β-glucan preparation. Although β-glucan was effectively extracted from cereal using this approach, McCleary demonstrated that successive water extraction at 40, 65, and 950C boosted the extraction rate and yield of barley β-glucan to 90% (Brennan and Cleary, 2005). Another study found that enzymatic extraction yields more than acidic or alkaline extraction, however protein was determined to be the predominant contaminant in this investigation (Ahmad et al., 2010).

Similarly, adding enzymes to the extraction process enhances the viscosity of dietary fiber by allowing β-glucan to be released from the food matrix, according to another study (Gamel et al., 2014).

2.6 Water Extraction

Grain samples were ground, screened for appropriate particle size, and refluxed with ethanol prior to extraction in the water extraction techniques described below. Refluxing in ethanol aids in the inactivation of enzymes like β-Glucanase as well as the elimination of the majority of lipids. Extraction at 47 to 50 °C prevents starch from becoming solubilized and then gelatinized. The MW of β-glucan obtained was influenced by the extraction temperature and cultivar type (Maheshwari et al., 2017).

Whole oat flour (<0.8 mm)

↓

Reflux in Ethanol (1:10) (80 - 82 % v/v, 2h, 85 - 90 °C)

↓

Supernatant removed, Residue washed twice with Ethanol (95%, v/v)

↓

Dried (40 °C, 12h)

↓

Aqueous extraction (1:8 - 1:10), 47-50°C, 2-3h

↓

Centrifuge (3000 - 4000 g, 10-15 min.)

↓

To supernatant 0.02% NaN_3 w/v

↓

Concentrate to ~1/3 v, 75 °C –vacuum

↓

Precipitate with twice volume of 95% Ethanol

↓

Filter the ppt., wash residue twice with 95% EtOH

↓

Discard ppt., Supernatant dried at 40°C, 12h

↓

Solubilize in water, Freeze dry

↓

Oat gum

↓

Solubilize 30 g oat gum extracted in 1L water, 90^0 C, stirring

↓

Adjust pH to 4.5 with 2.0 M HCl

↓

Precipitate protein at 25°C for 12h

↓

Centrifuge (3000 g, 20 min.)

↓

Adjust pH of supernatant to 7.0 with 2M NaOH

↓

Dialysis with double distilled water for 3 days

↓

Freeze dry

↓

Oat gum (βG 87-90%; protein 3-6.5%)

Figure 2.12 Flow chart for water extraction & purification of β-glucan from oat flour (Skendi et al., 2003)

The effect of extraction methods on the physicochemical and functional properties of β-G isolated from barley was examined by Ahmad and collaborators (2009). β-G was retrieved from barley using four distinct media: acidic, alkaline, hot water, and enzyme. They assessed the isolated β-G's yield, purity, and several physicochemical and functional properties. According to their findings, (i) the yield of β-G obtained by acidic extraction was lower than that obtained by hot water and enzymatic extraction but higher than that obtained by alkaline extraction, and (ii) of the four methods, hot water extraction gives the highest yield and purity, ensuring the maximum removal of impurities. In addition, this approach produced the most soluble β-G (Ahmad et al., 2009).

2.7 Acidic Extraction

Despite the fact that there are several reports on various extraction procedures for β-G, only a few workers have documented extraction under acidic circumstances. The contributions of Ahluwalia and Ellis are noteworthy (1984). They looked at how β-G and starch were determined in barley and malt. Barley samples were taken from trials in Ickleton, near Cambridge. They used a simple and quick approach to extract β-G and starch, using 50 mM perchloric acid. The extract was then utilized without drying to assess the amounts of β-G and starch in the extract solution.

By treating the extract with sodium acetate buffer and "treated cellulase," the extract was hydrolyzed to glucose for β-G measurement. The amount of glucose released was determined enzymatically using glucose-6-phosphate dehydrogenase, with the results expressed as β-G (%) on a dry weight basis (Ahluwalia and Ellis, 1984).

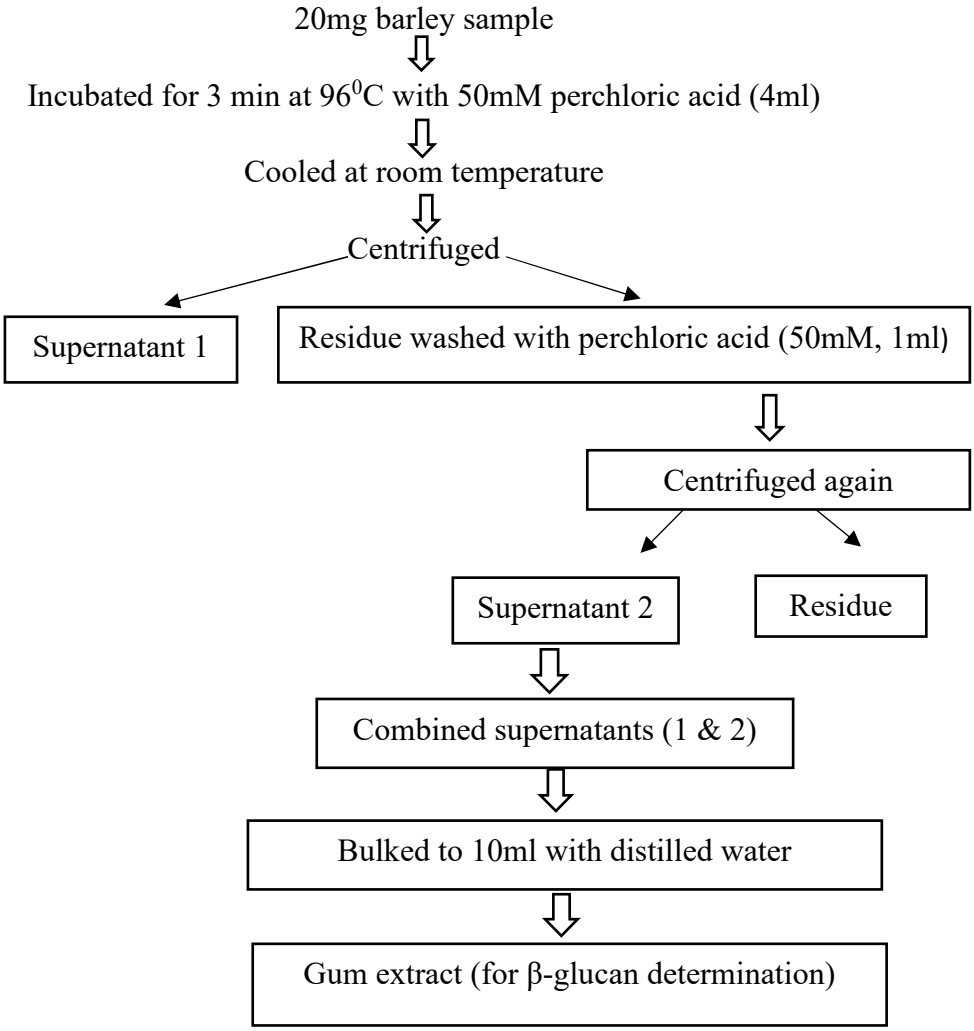

Figure 2.13 Acidic extraction scheme of β-glucan from barley (Ahluwalia and Ellis, 1984).

With a modified process, the yield of β-G from oat was extracted under acidic, alkaline, and enzymatic conditions, and it was discovered that the yield from acidic extraction was the lowest. They found that the oat β-glucan isolated under the aforementioned circumstances had a modest amount of protein, as well as major and minor components such as P, K, and Ca, as well as good water binding capacity (WBC), viscosity, and whippability qualities suited for industrial applications (Ahmad et al., 2010). When comparing alkaline and enzymatic extractions with defatted oat flour, acidic extraction yielded a lower yield of β-glucan (Babu, 2015).

2.8 Alkaline Extraction

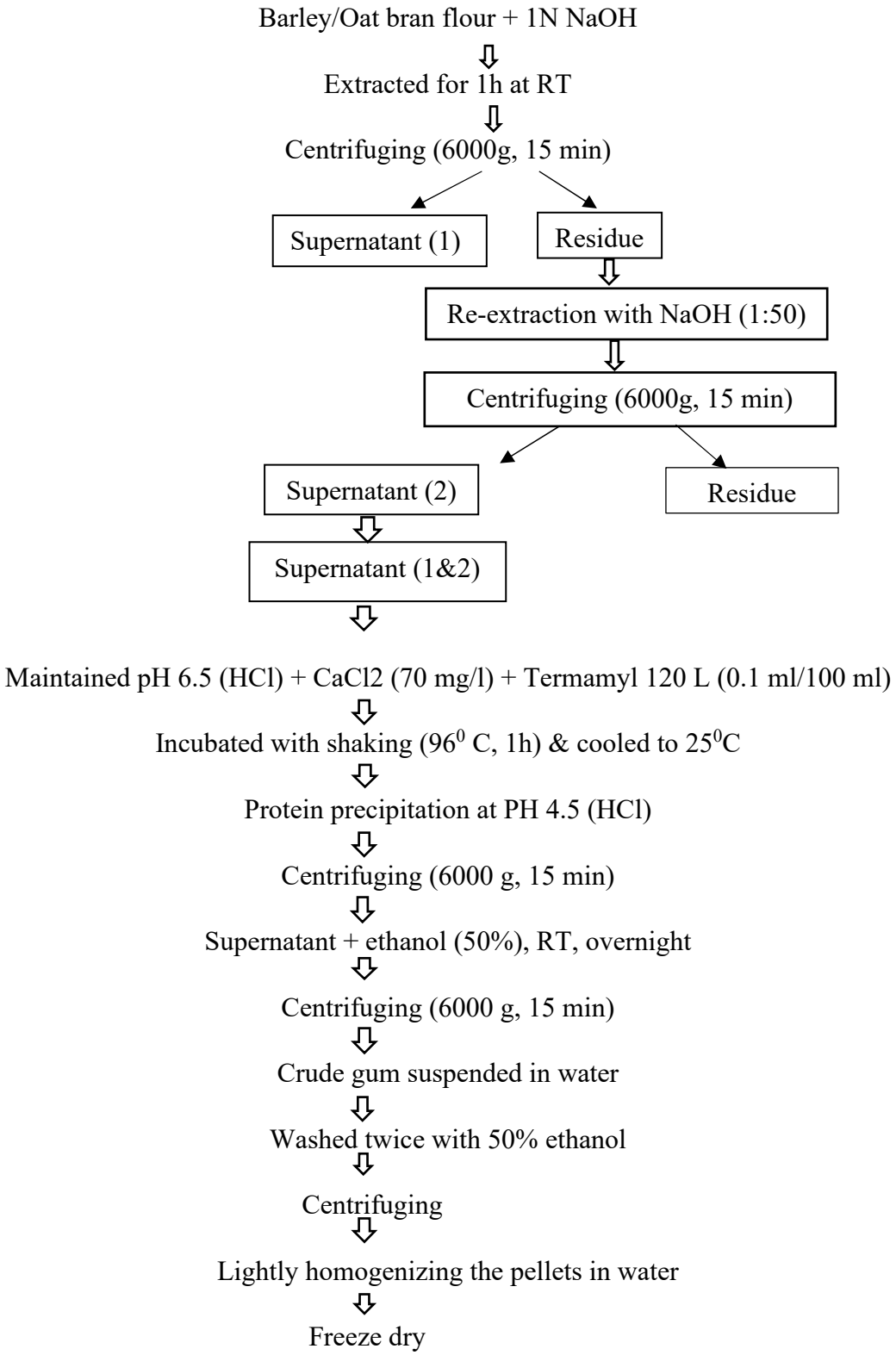

Figure 2.14 Alkaline extraction of β-glucan from oats and barley (Bhatty, 1995)

For the isolation of β-G from oat, Wood and colleagues (1978) used an alkaline extraction procedure and investigated the effects of particle size, solvent/sample ratio, temperature, and the effect of enzyme deactivation on the extraction yield. Among the various particle sizes used (3.0, 0.75, and 0.5 mm), oat flour with a particle size of 0.5 mm yielded a high yield of β-G. There was no significant difference in yield when the solvent/sample ratio was changed from 10:1 to 20:1. However, when the temperature was raised from 5 to 80 °C, the output increased from 3.41 to 4.59 percent db of flour. It was discovered that a temperature of 450C. was required to prevent starch gelatinization. While defatted oat flour yielded 3.78% β-G, flour with the enzyme deactivated yielded 3.5 percent β-G. Furthermore, the study found that this extraction procedure results in lower levels of β-G in barley flour samples than in oat flour samples. The viscosity of β-G produced from barley has likewise been found to be lower than that of oats. (Wood et al., 1978).

In 0.25, 0.5, 0.75, and 1.0 M NaOH solutions, β-G solubility was 77%, 88 percent, 98%, and 97 percent, respectively. At the laboratory scale, 1.0 M NaOH was employed throughout the extraction method, although 0.75 M NaOH was also found to be similarly effective. On the pilot plant scale, 0.25 M NaOH was employed to avoid handling dangers, and extraction was found to be as high as that seen in the laboratory with 0.75 M NaOH (Bhatty, 1995).

It's worth noting that several precipitations of β-G extract with ammonium sulfate yielded β-glucan with a high purity (91.58%). The structural analysis, MW distribution, and gelation properties of the extracted gum were investigated. Gelation kinetics demonstrated that as molecular size increased, the rate of gelation decreased. The entire procedure took 4 to 5 days (Li et al., 2006).

Recently, it has been demonstrated that the fungus β-G is beneficial in decreasing blood sugar and cholesterol (Zhu et al., 2015) as well as in fighting tumors and boosting body immunity (Du et al., 2015). In everyday practice, special consideration must constantly be given to the extraction of bioactive components from plant sources for herbal and therapeutic purposes. The efforts on qualitative extraction and quantitative separation of oat β-G are necessary for obtaining the full benefits of β-G, which demands its availability in high quantities (Maheshwari et al., 2017).

Chapter-3: Materials and Methods

3.1 Study area and Study period

The research work was conducted for six months from January, 2021 to June, 2021. Experimental procedures were carried out in the Dept. of Applied Food Science and Nutrition, Dept. of Food Processing and Engineering, Dept. of Animal Science and Nutrition, Dept. of Physiology, Biochemistry and Pharmacology at Chattogram Veterinary and Animal Sciences University, Bangladesh.

3.2 Layout of experiment

After collection of whole oat sample and after milling into 0.5mm on size, this flour was used for extraction of soluble β-glucan using the following extraction strategies (hot water extraction, alkaline extraction, acidic extraction) (Ahmad et al., 2009) with some modifications. Then β-glucan quantification was conducted through crude fiber content estimation and spectrophotometric analysis.

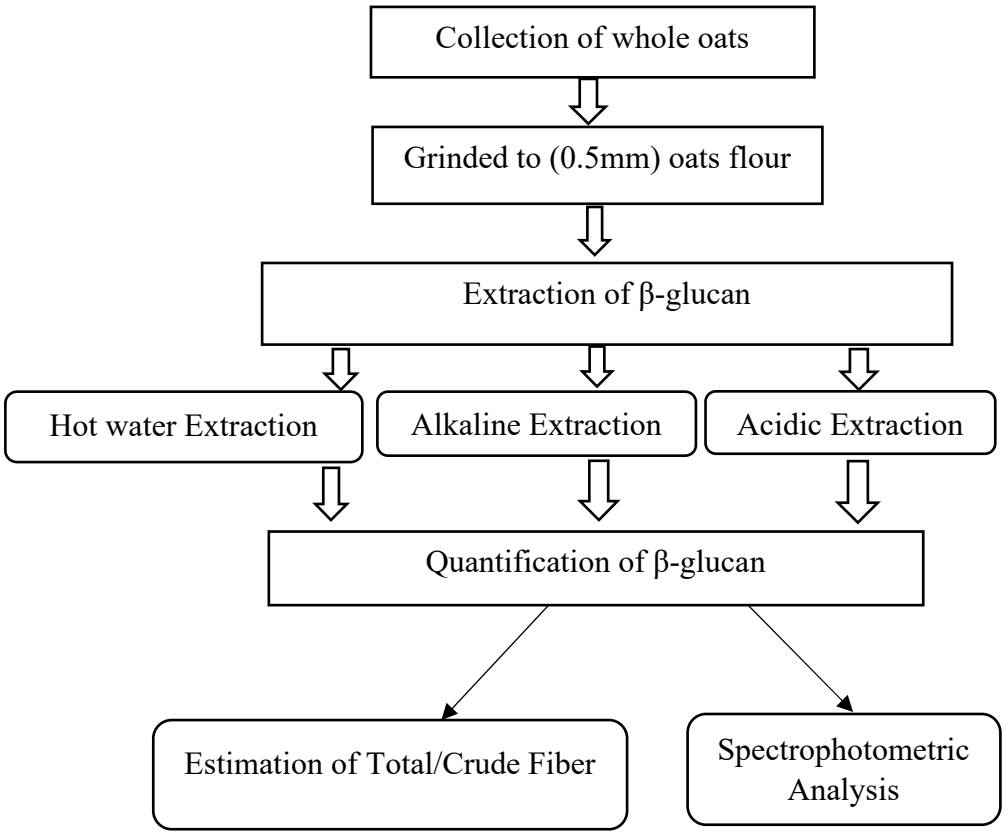

Figure 3.1 Flow chart of study design

3.3 Sample collection and preparation

Firstly, whole oats (*Avena sativa*) sample was collected from Khulshi area of Chattogram, Bangladesh. After collection of samples, it was milled for preparation of oats flour (0.5mm) from whole oats. Then the subsequent three methods (hot water extraction, alkaline extraction, acidic extraction) (Ahmad et al., 2009) were followed with some modifications for extraction of soluble β-glucan.

3.4 Hot water extraction of β-glucan

β-glucan extraction through hot water was done by following the hot water extraction method (Ahmad et al., 2009) with some modifications. The basic idea behind this procedure is to use hot ethanol to deactivate natural beta-glucanase enzyme and then hot water to extract the beta-glucan. To separate the proteins and fibers, the procedure employs a slightly alkaline solution and acid, and then adds ethanol to precipitate the extracted beta-glucan granules.

At first 100g oats flour (0.5 mm) was measured in a bowl & taken in a 1000 ml conical flask. Then 250 ml ethanol (80%) was poured in that flask and refluxed (evaporating + condensing) the solution for 6 hours by using Soxhlet apparatus. The main purpose of refluxing a solution is to heat a solution in a controlled manner at a constant temperature. After that the flour was mixed with distilled H_2O in 1:10 ratio and stirring the solution with magnetic stirrer at high speed for 90 min at 55^0C temperature. Then the mixer was centrifuged at 9000 rpm for 30 min at 40^0C by using 50 ml centrifuged cuvette. After centrifuging the supernatant (the liquid lying above a solid residue) was adjusted at pH 8.5 with sodium bicarbonate and stirred on hot plate at 55^0C for 30 min. Then the supernatant was centrifuged at 9000 rpm for 30min and then adjusted at pH 4 with citric acid and again centrifuged at 9000 rpm, 30 min. That time the supernatant was mixed with 80% ethanol in 1:1 ratio and hold for 20 min at room temperature and jelly like semisolid β-glucan (white color) was visible on the solution. Then this ethanol mixed solution was cooled at 4^0C & centrifuged at 4500 rpm for 15 min. β-glucan gum pellets was precipitated at the bottom of the centrifuged tube. Finally extracted β-glucan gum pellets were dried by using hot air oven.

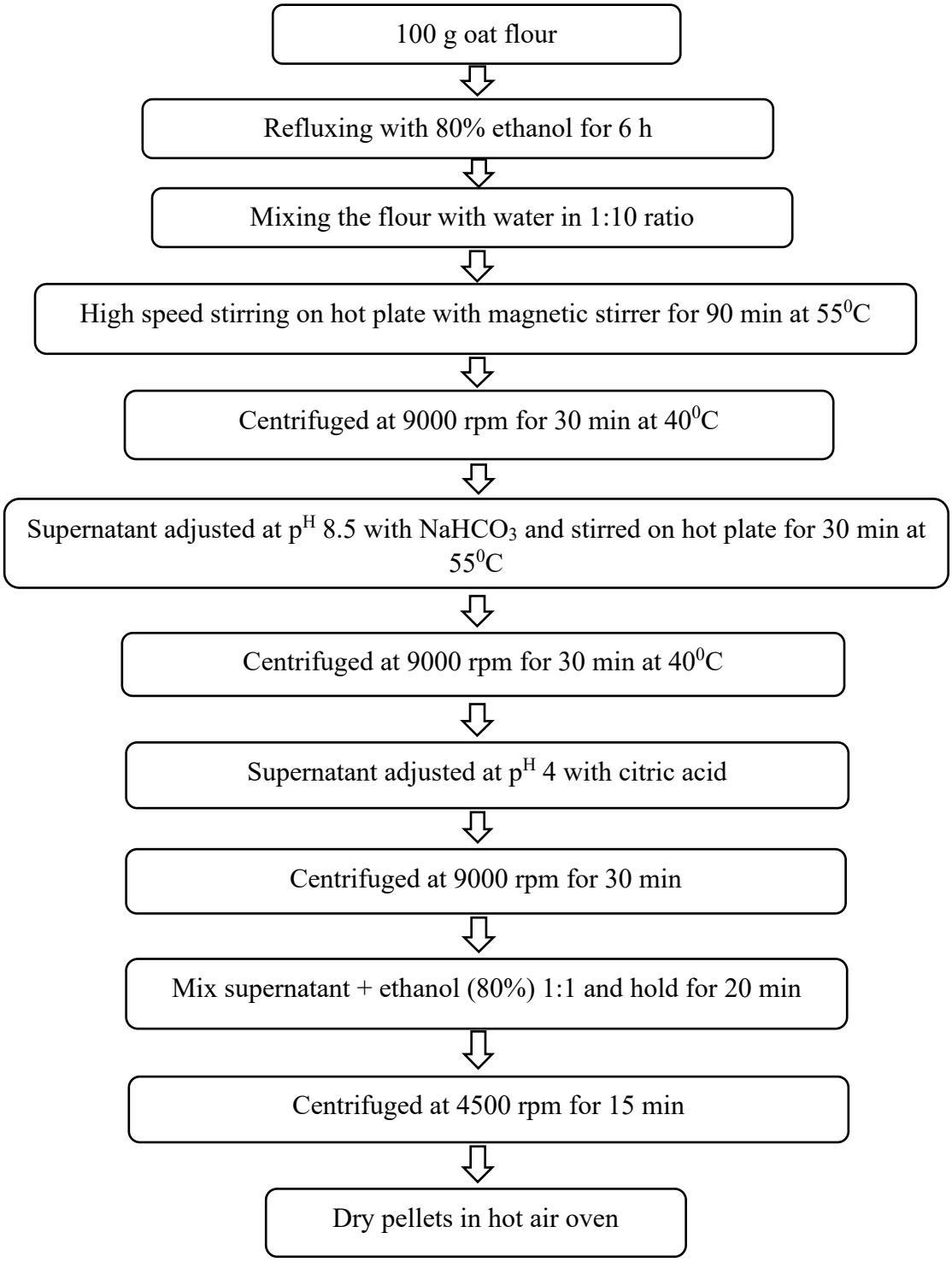

Figure 3.2: Flow diagram of hot water extraction procedures of β-glucan from oats

3.5 Alkaline extraction procedure of β-glucan

β-glucan extraction by using alkali as solvent was done by following the alkaline extraction method (Ahmad et al., 2009) with some modifications. In this process 1M Sodium hydroxide (NaOH) was used as alkaline solvent.

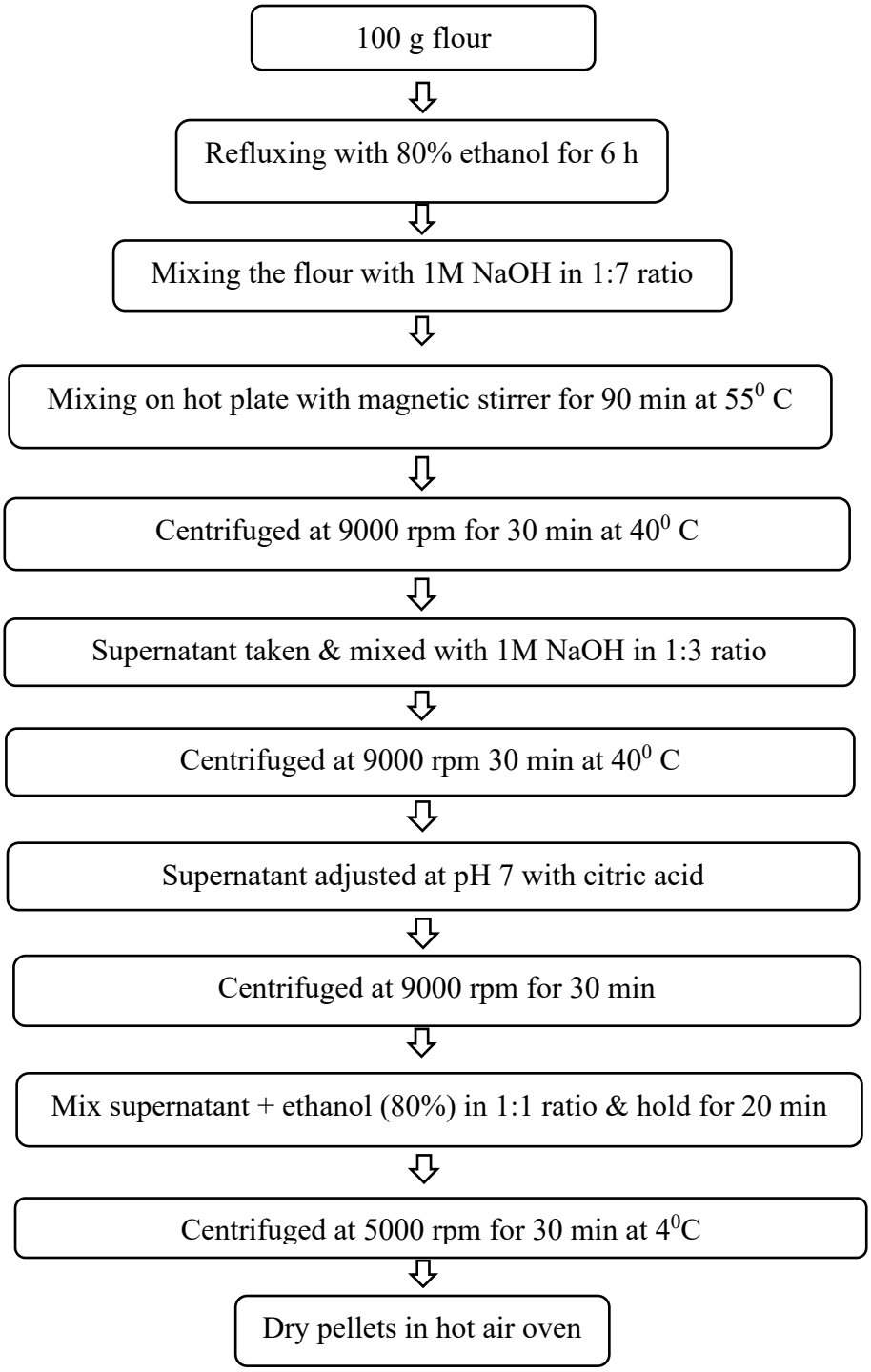

Figure 3.3: Flow diagram of alkaline extraction procedures of β- glucan from oats

Firstly, 100 g oats flour (0.5 mm) was taken in a bowl & transferred in a 1000ml conical flask. Then 250 ml ethanol (80%) was added in the conical flask and refluxed (evaporating + condensing) the solution for 6 hours by using Soxhlet apparatus. After that the flour was mixed with 1M NaOH in 1:7 ratio and mixing the solution with magnetic stirrer for 90 min at 55^0C temperature. Then the mixer was centrifuged at 9000 rpm for 30 min at 40^0C by using 50 ml centrifuged tube. After centrifuging the supernatant (the liquid lying above a solid residue) was separated and mixed with 1M NaOH in 1:3 ratio. The solution was centrifuged at 9000 rpm for 30 min at 40^0C temperature. Then supernatant was adjusted at pH 7 with citric acid and centrifuged at 9000 rpm for 30 min. That time the supernatant was mixed with 80% ethanol in 1:1 ratio and hold for 20 min at room temperature and β-glucan gum (white color) was floated on the ethanol solution. Then this ethanol mixed solution was cooled at 4^0C & centrifuged at 5000 rpm for 30 min. β-glucan gum pellets was precipitated at the bottom of the centrifuged tube. At last, extracted β-glucan gum pellets were dried by using hot air oven.

The extraction of β-G was more successful with NaOH than with Na_2CO_3 (Bhatty, 1993). Cui and colleagues (2000) reported the first isolation of β-G from preprocessed wheat bran using 1.0 M NaOH and neutralization with HCl. The pH of the starch digestion solution was adjusted with sodium acetate buffer, and the precipitated mass was resuspended in 2-propanol for purification. The traditional method of precipitation with $(NH_4)_2SO_4$ during the purification step was not effective in wheat, unlike in barley and oats. It was also discovered that as the pH is reduced from 7 to 4.5, the solubility of β-G increases.

3.6 Acidic/ solvent extraction of β-glucan

β-glucan extraction by using citric acid as solvent was done by following the acidic extraction method (Ahmad et al., 2009) with some modifications. In this process 1M citric acid ($C_6H_8O_7$) was used as solvent.

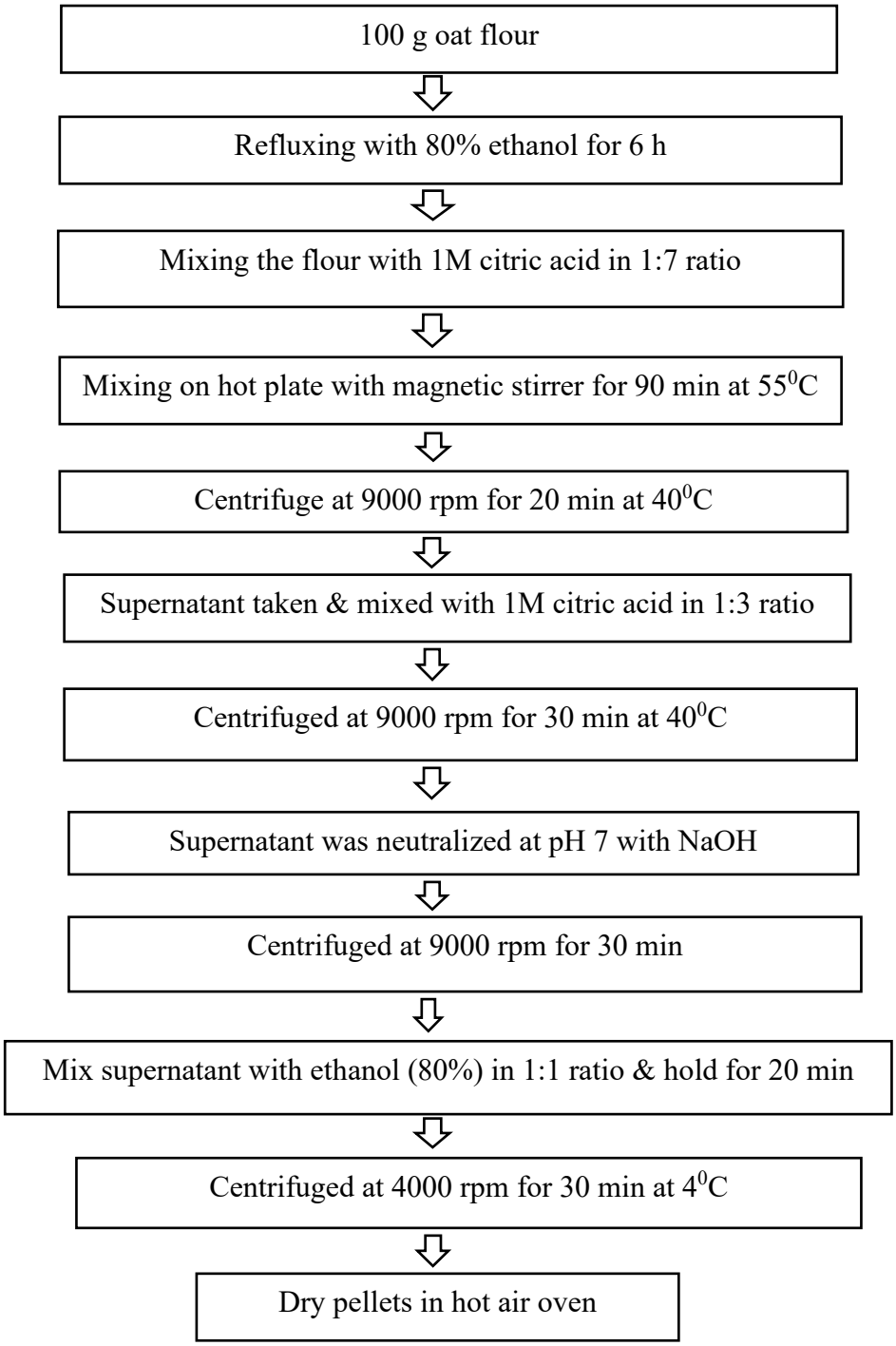

Figure 3.4: Acidic extraction schemes of β-glucan from oats

In the extraction process, firstly 100 g oats flour (0.5 mm) was weighed & taken in a 1000 ml conical flask. Then 250 ml ethanol (80%) was added with the flour to make a solution and refluxed (evaporating + condensing) the solution for 6 hours by using Soxhlet apparatus. After that the flour was mixed with 1M citric acid in 1:7 ratio and mixing the solution with magnetic stirrer for 90 min at 55^0C temperature. Then the mixer was centrifuged at 9000 rpm for 30 min at 40^0C by using 50 ml centrifuged tube. After centrifuging the supernatant (the liquid lying above a solid residue) was separated and mixed with 1M citric acid in 1:3 ratio. The solution was centrifuged at 9000 rpm for 30 min at 40^0C temperature. Then supernatant was adjusted at pH 7 with 1M NaOH and centrifuged at 9000 rpm for 30 min. That time the supernatant was mixed with 80% ethanol in 1:1 ratio and hold for 20 min at room temperature and β-glucan gum was floated on the ethanol solution. Then this ethanol mixed solution was cooled at 4^0C & centrifuged at 4000 rpm for 30 min. β-glucan gum pellets was precipitated at the bottom of the centrifuged tube. Finally, extracted β-glucan gum pellets were dried by using hot air oven.

3.7 Identification tests of β-glucan

3.7.1 Crude fiber

Crude fiber is the water insoluble fraction of carbohydrate consists mainly of cellulose, hemicellulose and lignin. It is estimated through digestion of fat free known amount of food sample by boiling it in a weak solution of acid (1.25% H_2SO_4) for 30 minutes followed by boiling in weak solution of alkali (1.25% NaOH) for 30 minutes at constant volume and then deducting ash from the residue obtained.

Electric balance, hot air oven, desiccator, metal tongs, crucible, muffle furnace, filter paper, pyrex beaker, round bottom condenser, measuring cylinder were used as apparatus. 1.25% (w/v) H_2SO_4 and NaOH solutions, 1% (w/v) HCl solution were used as reagents and N-octanol was used as antifoam.

At first 2 gm of the sample was weighed and then taken into a beaker. Then 125ml 0f 1.25% sulfuric acid solution and 3-5 drops of n-octanol were added as antifoam agent into the beaker. The beaker was boiled for 30 minutes at constant volume. After that, the sample was washed three times with distilled water to remove the acid. After washing 125ml of 1.25% sodium hydroxide and 3-5 drops of antifoam were added. It was again boiled for another 30 minutes at constant volume. The mixture was filtrated

and again washed the residue like before. It was washed again with 1% HCl solution in order to remove the acid. Then the residue was dried in a hot air oven at 105℃ until a constant weight was found out. It was placed in a desiccator for cooling and the weight was recorded. Finally, the residue was burned up to smoke and ignited in the muffle furnace at 550-660℃ for about 4-6 hours until that turned into white ash. The ash particles were weighed and calculated to determine the crude fiber content of the sample.

The limitation of the test is acid and base solubilize some true fiber particularly hemicellulose, β-glucan, pectin, lignin.

3.7.2 Spectrophotometric determination

A spectrophotometer is an instrument that measures the amount of light absorbed by a sample. The working principle of the Spectrophotometer is based on Beer-Lambert's law which states that the amount of light absorbed by a color solution is directly proportional to the concentration of the solution and the length of a light path through the solution. Spectrophotometry determines concentration of a substance in solution by measuring light absorbed by the solution at a specific wavelength.

This determination was conducted with the L-cysteine sulfuric acid method (Climova et al., 2021; Wood et al., 1977). For preparing β-glucan solution, 5 g extracted β-glucan gum was weighed in a weight balance and then it was dissolved in 1 ml distilled water.

Figure 3.5: β-glucan solutions

For the preparation of L-cysteine sulfuric acid solution, at first, 5 ml 86% sulfuric acid was prepared from 98% sulfuric acid. For that 4.4 ml sulfuric acid was added in 0.6 ml deionized water. Then 0.7g L-cysteine was weighed by a balance and dissolved in 1ml 86% sulfuric acid. Glucose standard solutions of different concentration were prepared by diluting 100 mg/dl glucose standard. 2 ppm, 50 ml glucose solution was prepared by dissolving 100 µl glucose standard in 50ml deionized water. 4 ppm, 50ml glucose

solution was prepared by adding 200 µl glucose standard in 50 ml water. For the preparation of 6 ppm, 50ml glucose solution, 300 µl standard was added with 50ml deionized water. And 8 ppm, 50ml solution was prepared by dissolving 400µl glucose standard in 50 ml deionized water.

Figure 3.6: Serial dilution of glucose standard

At first, 200 µl of the β-glucan solution was taken in a conical flask. Then the freshly prepared L-cysteine sulfuric acid reagent (1 ml) was added in the solution. The mixture was left for 3 min in a boiling water bath, then at the room temperature for 40 mins. The concentration of the pure β-glucan was measured by a spectrophotometer at wavelength 415 nm and with the glucose standard curve presented in Figure-3.7. Afterwards, known molecular weight of glucose, oat β-glucan, and the determined concentration of the β-glucan could be defined.

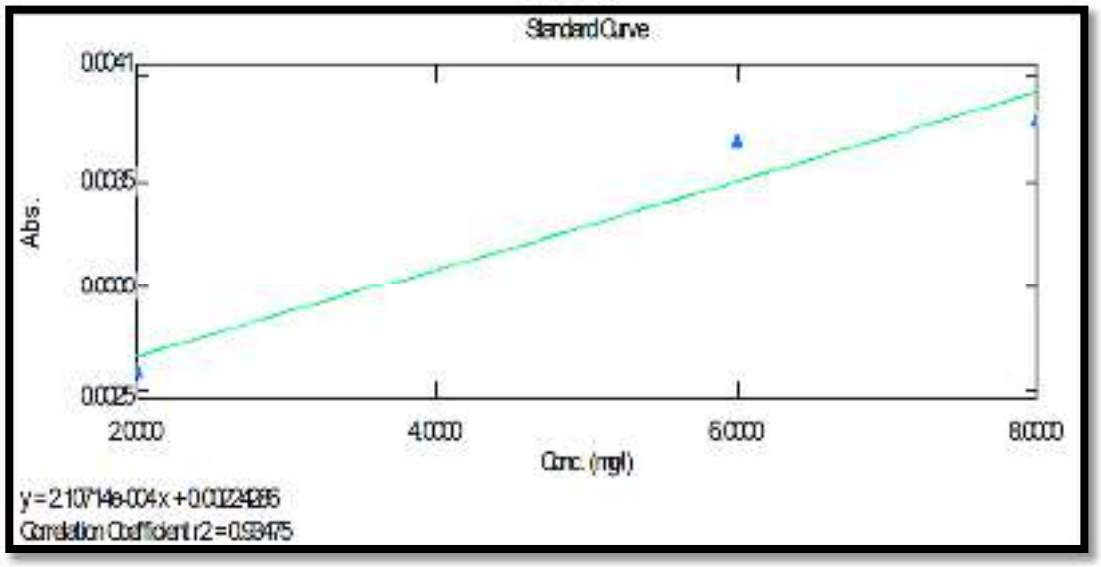

Figure 3.7: Glucose standard curve of extracted samples at wavelength 415 nm

The concentration of glucose was determined using the equation y = 210714e-004x + 0.00224286 with an R^2 value of 0.93475.

Chapter-4: Results

4.1 Extracted yield of β-glucan gum from oat

The amount of soluble dietary fiber called β-glucan gum that was obtained from 100 grams of oat flour is called the product's yield. Various extraction techniques produced gum yields ranging from 3.70 to 6.94 percent. From alkaline extraction, 6.94 g of pellets was yielded from 100 g of whole oats flour which represent 6.94% of extract. Similarly, from hot water extraction and acidic extraction, 4.54 g and 3.70 g of pellets were yielded from 100 g of whole oats flour which represent 4.54% and 3.70% of extract respectively. The alkaline extraction approach produced the highest yield (6.94%), followed by the hot water extraction procedure (4.54%), while the acidic extraction procedure (3.70%) produced the lowest yield.

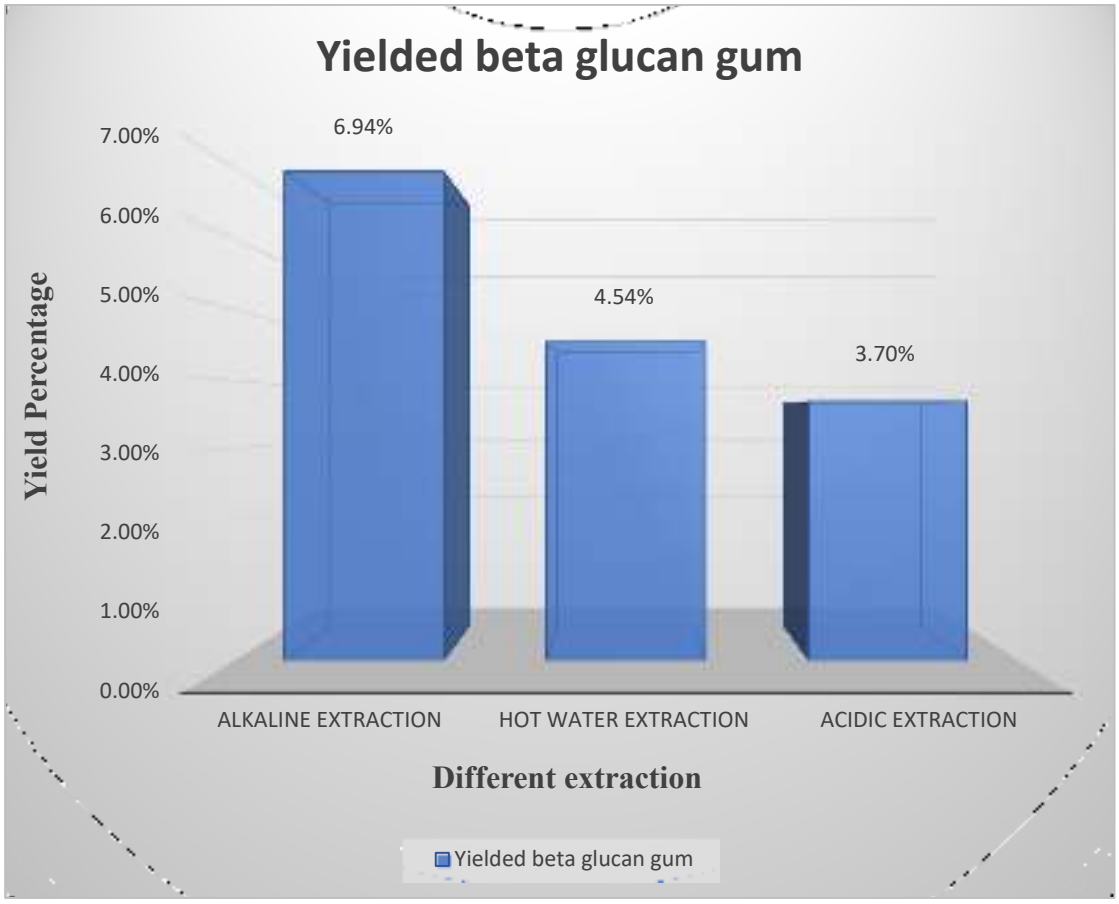

Figure 4.1 Comparative analysis of extracted β-glucan from oats (*Avena sativa*)

4.2 Identification of Crude fiber

Crude fiber represents the water insoluble fiber fraction of carbohydrate but β-glucan is the water soluble fiber fraction. The amount of indigestible substances like cellulose, pentosans, lignin, and other similar substances that are contained in food is measured as crude fiber. It is the leftover plant material from solvent extraction and digestion with weak acid and alkali. Although they have minimal nutritional value, these ingredients give the intestinal system the volume it needs to function properly.

In crude fiber estimation process, acid and base solubilize some of the true fiber particularly hemicellulose, pectin, β-glucan, lignin. The crude fiber content in yielded β-glucan gum samples of different extraction procedures ranged from 0.00% to 0.76%. Alkaline and acidic extracted gum pellets could not identify any insoluble fiber which represent that the extracted yields were soluble fiber. Hot water extracted yield contained the highest amount of crude fiber of 0.76%, whereas alkaline & acidic extracted samples contained the lowest (0.00%) crude fiber.

Table 4.1 percentage of crude fiber in different extracted yields

Yielded Sample	**Crude fiber (%)**
Hot water extraction	0.76%
Alkaline extraction	0.00%
Acidic extraction	0.00%

4.3 Spectrophotometric analysis

Quantification in spectrophotometer analysis & determination of extracted yields (β-glucan gum product) represent the absorbance of light by the concentrate in the β-glucan sample solution. Absorbance of β-glucan ranged from 0.15 to 0.80 in different extraction procedures. Highest absorbance of 0.8039 was obtained when alkaline extracted sample was used followed by 0.4630 when hot water extracted sample was used, whereas lowest absorbance (0.1469) was analyzed in acidic extracted sample. The correlation coefficient (r^2) value was 0.94 in spectrophotometric analysis.

Table 4.2 Absorbance in spectrophotometer of different extraction yield

Samples (Extracted β- glucan)	Concentration (ppm)	Absorbance	Weighing factor
Alkaline yield	3804.4746	0.8039	10^9
Hot water yield	2186.6441	0.4630	10^9
Acidic yield	686.5085	0.1469	10^9

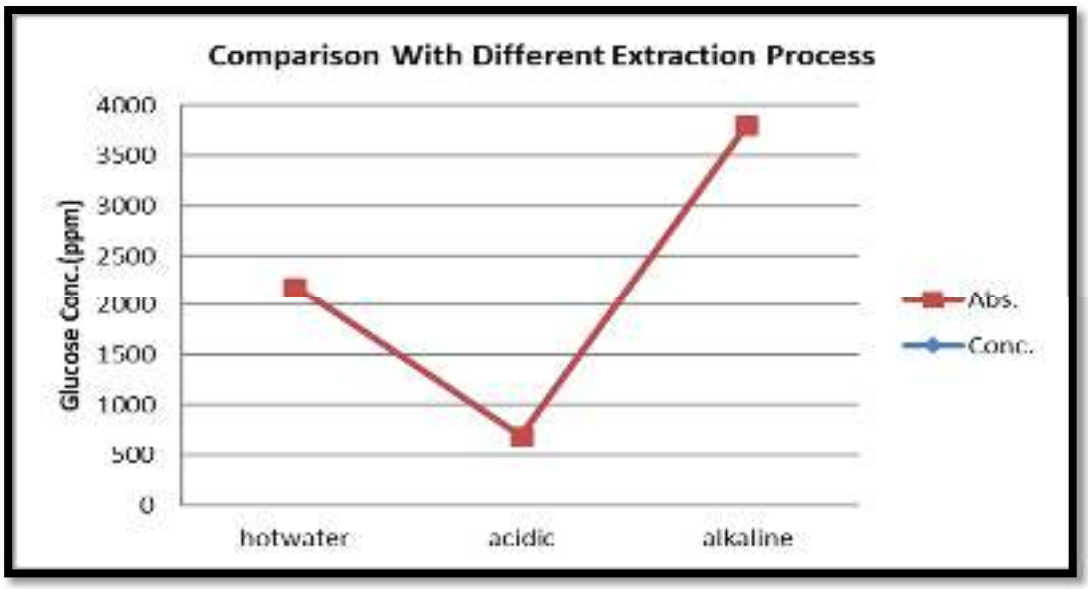

Figure 4.2 Comparative analysis of different extraction yields in spectrophotometer detection

Chapter-5: Discussion

5.1 Extracted yield of β-glucan gum

According to the aim of the present study three subsequent methods were performed for extraction of β-glucan (a soluble dietary fiber) from oat. Gum pellets obtained by respective methods were quantified through spectrophotometric analysis & crude fiber content estimation process. The weight of β-glucan gum obtained from 100 g of oat flour is referred to as the yield of gum product. Highest yield was found in samples that were extracted by alkaline treatment. Throughout the present investigation, a preliminary treatment of refluxing with 80% ethanol inactivated the β-glucanase enzyme, resulting in higher efficiency of alkaline extraction process. Another reason for the increased yield of β-glucan (6.94%) in the alkaline extraction procedure could be related to higher starch gelatinization and protein solubilization. Furthermore, the indigenous enzyme system was rendered inactive.

Different extraction processes yielded gum yields ranging from 3.94 to 5.40%. The highest yield of 5.4 percent was produced using the hot water extraction approach, followed by 5.22 % using the enzymatic extraction procedure, 4.65% using the acidic process, and 3.94% through using alkaline extraction procedure (Ahmad et al., 2009). Because of β-glucan cleavage by the β-glucanase enzyme, Symons & Brennan (2004a) noticed a reduced efficiency of hot water extraction. In other study, various extraction processes yielded gum yields ranging from 3.74 to 5.14 percent. When beta-d-glucan was extracted using an enzyme, the highest yield of 5.14% was obtained. Impurities such as fat, protein, starch, pentosans, and mineral (ash) matter were removed in addition to beta-d-glucan. The increased enzymatic recovery in that study was due to a preceding ethanol treatment and the use of appropriate enzymes that reduced the intermolecular interaction of beta-d-glucan with other oat components, resulting in enhanced beta-d-glucan extractability (Ahmad et al., 2010). A considerable difference between the yield results of the hot water extraction method (5.3 $\pm$ 0.3% in the dry state) and the acid extraction method can be seen in the yield of the β-glucan, which is equal to (4.4% in the dry conditions) (Ibrahim et al., 2017). Another study, the hot-water extraction method produced the largest yield of beta-glucan gum (5.4%), followed by enzymatic extraction (5.22%), acidic extraction (4.65%), and alkaline extraction (3.94%) (Climova et al., 2021). The physicochemical features of β-G can be

influenced by extracting it from the same cultivar under varied circumstances. The qualities of the final product are influenced by various parameters involved in the extraction conditions, such as pH, temperature, extraction time, centrifugal gravity and purification, and isolation procedures. A higher yield was observed when particle sizes of 0.5 mm (out of 0.5, 0.75, and 3.0 mm) were used, however a drop in yield was observed when fine flour was used. To avoid glucan destruction via hydrolysis, it is recommended that the innate β-glucanase enzyme be deactivated with ethanol treatment prior to the extraction method. The majority of the lipids are also removed during this procedure. At 80^0C, deactivation of innate β-glucanase is more effective than at room temperature. While non-solubilized starch must be extracted at a low temperature of 50°C, solubilized starch in extract can be eliminated by using a specialized enzyme. Protein can be eliminated by precipitation at pH 4.5 (optimal pH: 4 to 6), ammonium sulphate precipitation, or the use of a particular enzyme. Purification of β-glucan can be accomplished by centrifuging the extract multiple times and freezing the supernatant, or by precipitating with alcohol and vacuum drying the resulting precipitate containing β-glucan (Maheshwari et al., 2017).

5.2 Spectrophotometric Analysis

For the determination of the extracted yield and the recovery of the β-glucan extract, spectrophotometric analysis was performed. Spectrophotometry determines concentration of a substance in solution by measuring light absorbed by the solution at a specific wavelength. The percentage of glucose concentration in extracted β-glucan was highest in alkaline yield (0.38%) followed by 0.22% in hot water yield, whereas lowest 0.07% was observed in acidic yield and the absorbance of extracted β-glucan was measured in presence of glucose standard. High absorbance (0.8039) was shown by alkaline extracted yield followed by (0.4630) when hot water extract was used whereas lowest absorbance (0.1469) was analyzed in acidic extraction yield. The determined correlation coefficient (R^2) value was 0.94. Higher R^2 values represents smaller differences between the observed data and the fitted values. In the extracted pellets, there is a pure concentration of β-glucan. The L-cysteine sulfuric acid technique was used to get this determination. The specific measurement of glucose in oats gum was made using a cysteine sulfuric acid reaction with a beta glucan solution (wood et al., 1977). Using the L-cysteine sulfuric acid method, the concentration of β-glucan in the extract was determined (Climova et al., 2021).

In comparison to hot water and acidic extraction procedures, the percentage of glucose concentration was highest in alkaline extraction method.

5.3 Crude fiber

According to present study, hot water extraction procedure represents 0.76% crude fiber or insoluble dietary fiber (IDF) but in the other two extraction procedures (acidic & alkaline) the IDF was reported 0.00% and the extracted β-glucan gum pellet was soluble dietary fiber (SDF). The SDF range of β-glucan gum pellets found in a study was 74.11–76.85 percent (Ahmad et al., 2009). Another analysis revealed that the SDF of purified β-glucan concentrate ranged from 87.2 to 91.4% while that of unpurified concentrate was from 50.7 to 54.1% (Faraj et al., 2006). Arabinoxylan, arabinogalactan, cellulose, b-glucan, lignin, pentosan, and resistant starch are some of the most important components of dietary fiber. Soluble and insoluble dietary fibers are two types of dietary fiber. The soluble dietary fiber made up the majority of the β-glucan. These fibers help to lower the glycemic index by slowing the release of sugars (Cavallero et al., 2002). The hot water extraction method yielded the highest amount of soluble dietary fiber. When extracted with alkali, these gum pellets contain a small quantity of soluble fiber. At high pH and high temperature, the content of β-glucan was shown to decrease, according to the literature (Temelli, 1997). In comparison to insoluble dietary fiber, all of the approaches contained more soluble fiber. There was also a strong negative connection between soluble and insoluble fibers. The alkali extraction technique yielded the most insoluble fiber, but the water extraction approach reduces the amount of insoluble fiber material in gum pellets (Ahmad et al., 2009). Dietary fiber's relevance is demonstrated by the reduction of total and LDL cholesterol. It was calculated that 1 g of SDF from oats could reduce total cholesterol by 0.037 mmol/L (Brown et al., 1999). Extracted beta-d-glucan gum pellets have a higher nutritional fiber content, making them appropriate for a variety of industrial applications such as baking bread, cookies, and other cereal -based pasta products (Ahmad et al., 2010)

It was concluded that, though various extraction methods had a significant affect on extracted β-glucan gum yield and recovery, all the three extraction procedures can possible to perform in laboratory scale and in effective cost. If we compare the efficiency of the different extraction procedures in terms of β-glucan yield percentage, alkaline extraction yielded a higher yield and recovery of β-glucan.

Chapter-6: Conclusions

The method of extraction found to be crucial because it influenced the majority of the physiochemical parameters of extracted β-glucan gum. The alkaline extraction method was found to be successful because it produced maximum yield of beta glucan gum. The recovery and most of the physicochemical parameters of extracted β-glucan were significantly affected by different extraction procedures in this study. The yield and recovery of β-glucan were better with alkaline extraction. Extraction methods had an effect on the dietary fiber content of extracted β-glucan, according to the current research. All extraction methods used in this investigation have a high potential for producing β-glucan gum based on the extracted yield. On a cumulative basis, however, β-glucan extracted using the alkaline extraction method seems to be a simple procedure for a laboratory scale or pilot plant project, as well as a promising additive with a lot of potential for use in food items. The study revealed that β-glucan extracted using the alkaline extraction method has both scientific and economic relevance. Researchers are paying close attention to the growing trend of using nutritional food products with a variety of health benefits for various segments of the population. β-glucan is at the top of the list because of its numerous health benefits, vast range of industrial applications ranging from food to cosmetics and medicine, and availability from a variety of sources including plants (cereals), fungi, and bacteria. Anti-diabetic, anti-constipation, hypolipidemic, anti-cancer, and other health benefits of β-glucan are just a few of them. Due to rising medication costs and side effects, consumers are becoming increasingly interested in making dietary changes to improve their health. It is a well-known truth that oats can help with a variety of current lifestyle disorders.

Chapter-7: Recommendations & Future perspectives

Future research on the utilization of oat beta-glucan as a functional food ingredient is absolutely warranted. This investigation has shown a number of areas that need to be investigated further. Beta glucan interactions with other dietary components, in particular, are essential for increasing its application in the production of food and other unique products. Its uses in food range from baked foods to dairy to the meat industry. It's been employed in filmmaking, pharmaceuticals, and cosmetics. In a summary, we can say that β-glucan ushers in a new era in nourishment for humans, and scientists will continue to investigate its numerous beneficial properties in the future. The bioavailability of antioxidants from oat, as well as their impact on human health, are both urgently needed. It may be concluded from this article that β-glucan is the key active element responsible for all of oats' organoleptic and nutritional benefits. Because oats are a convenience cereal, further research is needed to justify and validate their nutraceutical status in geriatric and paediatric diets. New functional metabolites in oat that can be integrated in food products will require more research and development. It will be fascinating to learn how β-glucan could be employed in the identification of innovative products for use in our daily diets, as well as the impact of various processing and packaging technologies on β–glucan and its interactions with other food matrix components. Cardiovascular disease, obesity, and diabetes are all considered worldwide issues. As a result, oat-based products must be developed to meet the expanding nutritional and health needs of the population.

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Appendix A: Photo Gallery

Appendix A1: Pictorial presentation of soluble β-glucan extraction

Oat flour

Refluxing

Magnetic stirring

Centrifuging

Supernatant

p^H adjustment

Stirring supernatant

Centrifuging at 5000 rpm

Ethanol addition

Separated β-glucan (Alkaline)

Floated β-glucan

β-glucan gum pellet

Appendix A: Photo Gallery

Appendix A2: Pictorial view of β-glucan quantification

Serial dilution of glucose standard for spectrophotometric analysis

Placing in hot water bath

β-glucan solution preparation

Prepared β-glucan and glucose standard

Appendix B: Table of glucose equivalents of the samples

Glucose standard	Concentration (ppm)	Absorbance	Weighing factor
Standard 1	2.0000	0.0026	1.0000
Standard 2	4.0000	0.0032	1.0000
Standard 3	6.0000	0.0037	1.0000
Standard 4	8.0000	0.0038	1.0000

Brief Biography

Susmita Roy passed the Secondary School Certificate Examination in 2011 from Feni Govt. Girls' High School, Feni, and then Higher Secondary Certificate Examination in 2013 from Joynal Hazari College, Feni. She completed her B.Sc. (Hon's) in Food Science and Technology from the Faculty of Food Science and Technology at Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. Now, she is a candidate for the degree of Master of Science in Applied Human Nutrition and Dietetics under the Department of Applied Food Science and Nutrition, Chattogram Veterinary and Animal Sciences University (CVASU). She has an immense interest to work in improving health status of people through proper guidance and suggestions and to create awareness among people about food safety and nutrition.