ESTIMATION AND PROFILING OF BETA GLUCAN IN SELECTED MILLETS

By

A.AMREEN FATHIMA

(17PFD004)

A THESIS SUBMITTED TO

AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN

COIMBATORE – 641043

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE MASTER'S DEGREE IN

FOOD SERVICE MANAGEMENT AND DIETETICS

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Saradha Ramadas 22.04.2019.

SIGNATURE OF THE HEAD OF THE DEPARTMENT

22/4/19

SIGNATURE OF THE SUPERVISOR

ACKNOWLEDGEMENT

The investigator exalts **God Almighty** for being her refuge and strength and praises him for his everlasting love, bountiful mercy and amazing grace showered on her thought the study.

The investigator pays her reverential homage to **(Late) Dr.T.SAVINASHILINGAM,** Founder, and First Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for being a perennial source of inspiration.

Reverential homage is paid to the Doyen of Nutrition Colonel and **Dr. (Mrs) RAJAMMAL P. DEVADAS,** Former Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for being a perennial source of inspiration.

The investigator is grateful to **Padmashri Dr. P.R.KRISHNAKUMAR,** Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing an opportunity, infrastructure and all amenities to conduct the study.

The investigator expresses her gratitude to **Sri.T.S.K.MEENAKSHI SUNDARAM, M.A.,M.Phil.,Ph.D.** Managing Trustee, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, forproviding an opportunity to conduct the study.

The investigator owes her special thanks and gratitude to **Dr. (Mrs) PREMAVATHY VIJAYAN, M.Sc., M.E.d, Dip. Spl. Edn.(U.K), M.Phil, Ph.D,**Vice Chancellor, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for facilitating the completion of the study.

She records her gratitude to **Dr. (Mrs). S.KOWSALYA, M.Sc., M.Phil., Ph.D**., Registrar, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for providing all the facilities for the smooth conduct of the study.

She expresses her heartfelt thanks to **Dr. (Mrs).N.VASUGI RAJA, M.Sc., M. Phil, Ph.D.,** Dean School of Home Science, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, for her help and constant support to carry out the research.

The investigator expresses her special thanks and sincere gratitude to **Dr. (Mrs) R. SARADHA RAMADAS, M.Sc., MBA., M. Phil., Ph.D.,** Professor and Head, of the Department of Food Service management and Dietetics, Avinashilingam Institute for Home Science and Higher education For Women, Coimbatore, for her keen interest, valuable help, concern and encouragement which has helped in the successful completion of the study.

The investigator expresses her deep sense of gratitude and whole hearted thanks to her supervisor **Dr. (Mrs). R.RADHA, M.Sc., M. Phil., Ph.D.,** Assistant Professor, Department of Food Service Management and Dietetics , Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, whose ability to probe beneath text was a true gift and her insight have strengthened this study significantly. Her meticulous guidance, deep concern, constructive suggestions, continued motivation, enduring support and gentle care rendered from the initial to the final level of the study enabled the investigator to complete the research successfully.

The investigator acknowledges with gratitude the good wishes and encouragement of **all the staff members of the Department of Food Service Management and Dietetics,** Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

The investigator acknowledges her heartfelt gratitude to the Institutional Human Ethical Committee Members of Avinashilingam Institute for Home Science and Higher Education for Women Coimbatore, for approving her research work.

The investigator expresses her heartfelt thanks to **family, friends and relatives** whose motivation**,** care, concern and support for all their support morally, spiritually and financially for whom they are the pillar of strength.

The investigator in final but not the least gracefully acknowledges to all the people who had extended their unforgettable support and unfailing help throughout the research.

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I. INTRODUCTION

Millets are a range of cereal crops grown around the world which are used as grains in human food or fodder. They form a functional/ agronomic group but not taxonomic. (Shadang *et al*, 2014).

Millets are Indian traditional food. In the earlier days, our ancestors have been consuming millets. But as the years passed by, this tradition faded away. And now due to more of health awareness, millets have again emerged into the market and our food habits.

They are pearl (most cultivated variety), foxtail, Proso and finger millets. Millets grow better in dry, infertile soils and thus they are most adaptable to extreme conditions like high temperature, low & erratic precipitation, acidic and infertile soils with poor water-holding capacity (Shadang *et al*, 2014)

Millet grain have astounding benefits as drought resistant crops and are capable of growing and produce good yield even in the areas of water scarcity. They mainly gained importance due to its high nutritional values, low cost production, and ease of processing and manufacturing.

In India there is massive production of especially eight millets species namely Sorghum, pearl millet, finger millet, foxtail millet, proso millet, kodo millet, banyard millet and little millet (Dayaka*r et al, 2017).*

Millets are reffered to both cereal as well as the plant that produces these millet grains. Even today millet is observed as the staple food in most parts of India. Actually, four types of millets are cultivated in large scale (Shadang *et al,2014).*

According to FAO production data of 2016, sorghum ranks fifth among the major cereals after maize, paddy, wheat and barley. Every millet has a vernacular name. Finger millet (*Eleusine coracana*) is widely cultivated in India.

Millets are inevitable crops of Asia and Africa with 97% of production (Donough *et al,*2000). Millet have been cultivated for more than 10,000 years in the eastern part of Asia. India is recorded to be the top most producer of millets (Lu *et al*, 2009*).*

Although India is the largest producer and the largest consumer of millets with more than 40% of the world millet consumption, they have never been propelled to the top of the food and farming policies of the country (Konapur *et al,* 2014),

Millet oil can be a good source of fatty acids such as linoleic acid and tocopherols and they ar also rich in phytochemicals and micronutrients which benefit the health as they protect the immune system. Millets are considered nutraceutical due to the presence of antioxidants which in turn prevents hypotension, cardiovascular disorders diabetes and cancer. Other such health benefits are that, they take a long for gastric emptying and gives sateity and comprises of a variety of complex fibres. Most of all it is also gluten free. Even today millets are under estimated to be used only as cattle feed and this is mainly due to lack awareness. (Yang *et al*, 2012*).* And they are potent enough to fight against malnutrition (Devi *et al*, 2011).

Millets serve as the major source of nutrition in less afluent groups. A variety recipes can be made out of millets such as chapathi, bread, dosa, idly, porridge, savouries, sweets and cakes (Chandrasekara *et al, 2012)*

Millets are being emphasised due to its rich nutritional contribution , it provides 60-70% of carbohydrates, 7-11% of proteins, 1.5-5% of fat, 2-7% of crude fibre and also equally rich in vitamins and minerals. Millets are a very good source of vitamin-B, magnesium, several antioxidants and also an excellent source of dietary minerals like phosphorous, manganese and iron.(Singh *et al,*2012*)*

Millets also have a reserve of fatty acids such as behenic acid, arachidic acid, erucic acid. Millets are capable of producing energy as it contains niacin, folacin, riboflavin and thiamine (Adamou *et al*, 2011).

Millets are alkaline by nature, which when consumed and digested it mixes along with the digestive enzymes and maintain an optimum pH balance in the body and hence prevents diseases (Shobana *et al,*2009*)*

Even while vast segments of people suffer from undernutrition, particularly micronutrient deficiencies, there is a growing incidence of obesity and chronic diseases like diabetes, cardiovascular diseases, cancer etc. Both the ends of this grim spectrum are at least partly due to changing food habits and loss of millets from the diets is being considered as one of the important reasons. (Archana *et al*, 2014)

Eradication of extreme poverty and hunger is the first of the Millennium Development Goals (MGDs) proposed by the United Nations in the year 2000. India is far away from achieving this goal (MGD, 2010). India is among the 17 hunger alarming countries identified and ranked 65th with a score of 22.9 in Global Hunger Index (2012)

Millets have a potential to assume significance not only for food security but also for nutritional security in India, because of their hard nature and ability to grow in rain-fed lands with very little agricultural inputs and richness of many nutrients as compared to most of the cereals. (Konapur *et al*,2014)

According to the National Academy of Agricultural Sciences , 2013 the Initiative for Nutritional Security through Intensive Millets Promotion (INSIMP) aims to demonstrate improved production and post-harvest technologies in an integrated manner by promoting cultivation and consumption of millets and millets-based products. For this purpose, the Government of India announced an allocation of Rs. 300 crores in the budget of 2011-12, under Rashtriya Krishi Vikas Yojna (RKVY) .

Effect of processing on availability of nutrients is done in order to suggest the way forward to use millets in addressing food and nutrition security in India. (Rao *et al*, 2014)

Satisfaction of hunger is the primary criteria and therefore cereals are central to the issue of food security. Cereals and millets are rich sources of calories and other nutrients. Among food groups, if looked at the distribution of nutrients such as iron, about 50 per cent of it comes from cereals and millets (Gopalan *et al*, 1991).

At present, small millets account for less than 1 per cent of food grains produced in the world (ICAR, 2010). Although the contribution of coarse grains to total Decline in consumption of millet food grains has declined from 29 per cent in 1961 to about 12.5% during 2011-12, India still remains the leading producer of small millets namely, finger millet (Ragi), kodo millet (Varagu), foxtail millet (Thinai), barnyard millet (Kuthiravali), proso millet (Panivaragu), little millet (samai) as well as major millets like pearl millet (kambu) and great millet (cholam) (Majumder *et al,* 2006). Among millets, pearl millet ranks first in India, in terms of area, production and productivity. However, their major use as food has remained only in the areas where they are cultivated and to the traditional **preparations (Amodou *et al,* 2011).**

Nutritive value of millets and comparison with rice and wheat Among the millets, pearl millet has highest content of macronutrients, protein, fat and micronutrients such as iron, zinc, magnesium, phosphorus, folic acid and riboflavin. Finger millet is a good source of energy, calcium, phosphorus, magnesium, potassium and thiamine (Antony *et al,* 1996)

(Rao *et al,*2014) highlights that historically, millets have been marginalized both in policy and priorities of agriculture and nutrition in India. Millets can perhaps play a pivotal role in promoting nutrition security. They are often referred to as 'nutri-grains' since they are rich in micronutrients like minerals and B-complex vitamins. Despite these attributes, millets are losing their pride of place in production and consumption in India. In recent years, there has been some effort towards reviving millets.

Millets contain 37-38% (Glew *et al.,* 2008) of dietary fibre and it was earlier considered to be an anti-nutrient but now its approved to be a nutraceutical (Hegde and Chandra, 2005). Hence it's a complete food favourable for large scale consumption as snack, processed food, baby food etc.

The soluble sugar content of caryopses changes during development and is maximum 5.2% (Murthy *et al.*, 1985). Dietary fibre content of various Indian foods have been estimated (Rao, *1988*:NIN, 2017).

The studies conducted by (Easwaran *et al,* 1991; Kavitha *et al,* 2001) reveals that drietary fibre has 2 main benefits such as swelling properties and increased travel time in the small intestines. Especially sorghum and finger millet eliminates the risk of inflammatory bowel diseases. Millets are a great form of low calorie / calorie defecit diet as they have the water absorbing and swelling/ bulking properties. And also that sorghum, finger and pearl millet are apt for a diabetic diet as they have the property of absorbing carbohydrates slowly and therefore manages / treats impaired glucose tolerance. They also have the property of binding with bile acid and steroid there by reduces/ decreases the risk of cardio vascular disorders by promoting hypocholestero- laemic effect.

Millet are a great source of fibre as their bran is known as complex unavailable polysaccharide. They play a major role in reducing blood glucose levels and enhanceing insulin response due to its higher / increased viscosity,glycemic index and water holding capacity. Jowar is proven to reduce blood glucose levels among diabetic patients (NIDDM) (Kamble and Shinde, 2004)

Beta glucan is a functional component that naturally occurs in the cell walls of cereals, fungi and bacteria. Depending on the source its physiological properties will change. Oats is rich in β-glucan, but oats is not our native food. Cereal grains have different fibre contents. In cereal grain, a majority of fibre is found (at a decreasing rate) from the outer coat to the endosperm. This does not apply to arabinoxylan, which is a principal component of the endosperm cell wall. The methods of the fibre isolation and purification as well as the techniques implying quantitative and structural analyses have been developed to isolate the fibre from the common cereal fractions obtained in the grinding process. The elaborated procedure of the cereals processing (grinding) makes it possible to obtain products from the particular structural grain layer (Bressani *2003*; Lambo *et al*, 2005)

The physical and physiological properties of cereal β-glucans are of commercial and nutritional importance (Mälkki & Virtanen 2001*)*

β-glucans are indigestible polysaccharides occurring naturally in various organic sources such as corn grains, yeasts, bacteria, algae. They are important components of the fibres containing unbranched polysaccharides consisting of β-d-glucopyranose units linked through (1→4) and (1→3) glycosidic bonds in cereals and (1→6) glycosidic bonds in fungal sources, respectively. The structure has an impact on the water solubility of β-glucans. Extensive research has been done into the structure and properties of watersoluble β-glucans in contrast to water-insoluble β-glucans (Johansson *et al*, 2000; Ren *et al*,2003).

Generally, no sharp distinction exists between the soluble and insoluble fractions and the ratio is highly dependent on the extraction conditions of the soluble fibre (Virkki *et al. 2005*). Glucans are usually concentrated in the internal aleurone and subaleurone endosperm cells walls (Charalampopoulos *et al,* 2002*;* Demirbas 2005; Holtekjolen *et al*, 2006)

Millets also contain β-glucan in various amounts. And millets is our regional food. This study is conducted to estimate the β-glucan levels in various kinds of selected millets and thereby increase its rate of consumption. β-glucan is proven to improve heart health and also has other health enhancing properties. Beta glucan is a dietary fibre found in large amount in cereal grains such as oats, barley .Beta glucan has a lot beneficiary health properties and more over it is an inexpensive byproduct and hence can also be fortified. This fibre is present in the internal aleurone and sub aleurone cell walls (Wang *et al*, 2002)

Beta glucan is being in limelight for the past few decades due to its exceptional health promoting functions and bioactive properties (Ripsin *et al*,1992). And there are no adverse side effects reported due to consumption of high fibre diet (Hallfrisch *et al,*2003).

Glucans are glucose polymers categorised based on their interchain linkage either being α-or β-linked (Barsanti *et al*, 2011). They are a heterogenous group of nonstarch polysaccharides, containing of D-glucose monomers linked by β-glycosidic bonds (Wood *et al*, 2007).

The quality and quantity of beta glucan depends on the environmental conditions during the endosperm development. And for this licheninase is responsible which aids in cell wall rupture during germination (Stuart *et al*,1987).

(Wasser *et al*, 1999) observed that the highest amount beta glucan recorded in cereals are in oats 3-8g and barley 2-20g and other cereals also contain beta glucan in commendable amounts such as sorghum 1.1-6.2g, maize 0.8-1.7, rice 0.13g.

Hence the study was put forward with the ;

PRIMARY OBJECTIVE

- To know the importance and health benefits of beta glucan and to estimate the content of beta glucan in various millets that are commonly used.

SECONDARY OBJECTIVE

- To estimate the beta glucan content in various millets in laboratory using enzymic methods

- Identifying the millet/millets rich in beta glucan content

II. REVIEW OF LITERATURE

The review of literature for the study entitled ***"Estimation and Profiling of β- glucan in Selected Millets"*** was collected under the following topics.

A. Millets and its Production
B. Consumption of Millets in India
C. Types of Millet
D. Health Benefits Of Millets
E. Role of Millets in Non-Communicable Diseases
    a. Millets for the Management of Diabetes
    b. Millets for the Management of Cardiovascular Diseases
    c. Millets for the Management of Celiac Disease
    d. Millets as Antinutrients
F. Antioxidants in Millets
G. Classification of dietary fibers
H. Beta Glucan and Its Properties
    a. β-Glucan Definition
    b. Physiological effect of β-Glucan
    c. Source and Isolation (Oats)
I. Potential Health Benefits of Beta Glucan
    a. Effect of β-Glucan in Heart health
    b. Effect of β-Glucan in Immunity
    c. Effect of β-Glucan in Dyslipidemia
    d. Cholesterol reducing Mechanism
    e. Effect of β-Glucan in insulin resistance
    f. Effect of Oat β-Glucan in Gastrointestinal tract
    g. Effect of β-Glucan in blood pressure
    h. Effect of Oat β-Glucan in prevention of cancer
    i. Effect of Oat β-Glucan in Antimicrobial and Immune response
    j. β-Glucan in Functional food
    k. Antioxidant effect of β-Glucan

J. Physio-Chemical Characteristics of β-Glucan

a. Determination of β-Glucan
b. Sensory attributes of
c. β-Glucan content of various cereals
d. Digestibility of β-Glucan
e. Comparison of dietary components in cereals & legumes
f. Degrading enzymes from finger millet
g. Effect of fermentation on β-Glucan
h. Water solubility of β-Glucan
i. Compound responsible for β-Glucan degradation
j. Hydrolysis of β-Glucan

A. Millets and its Production

Millets are small in size , ball shaped and minor cereals which belong to *Poaceae* family.It is very much significant as it can grow in infertile soil, drought resistant, resistant to pests and crop related diseases (Devi *et al*, 2011) and grown all round the year and all over the world(Shahidi *et al*,2013).

Millets are major crops of Asia (especially in India),with 97% of millet production in developing countries. By the 2000s, the annual millet production had increased in India, yet per capita consumption of millet had dropped about 50 to 75%. (Basavaraj *et al,* 2010)

Millets are major food crop in developing countries as they consist of major and minor nutrients in commendable amount. Millets are potential enough to treat or cure non communicable diseases like Obesity, Diabetes mellitus , Cardio vascular disorders and they are also gluten-free food, which makes them suitable for celiac patients. (FAO, 2012).

B. Consumption of Millets in India

India is recorded to be the largest producer of Pearl millet (Bhattacharjee *et al,* 2007). Millets are exclusive since its rich in protein, calcium, dietary fiber, β-glucan and polyphenols and also contains essential amino acids that has sulphur such as methionine and cysteine (Obilana *et al*, 2002).

According to National Nutrition Monitoring Bureau , the consumption of millets is higher in the states of Gujarat (pearl millet, maize) Karnataka (finger millet), Maharashtra (sorghum) but comparatively very low in the states of Kerala, Orissa, West Bengal and Tamil Nadu since Rice is the most consumed cereal (NNMB, 2006). The major source of energy in Indian diet (70-80%) is obtained from cereals as it is their staple food (Gopalan *et al, 2009*). A recent study conducted by NNMB reveals that millet contributes only 2% of the total calories (6.7g/d) (Radhika *et al*, 2011)

C. Types of Millet

Millet can roughly be divided into four different types i.e. Pearl Millet (Pennisetum glaucum),Finger Millet,(Eleusine coracana), Foxtail Millet (Setaria italica) and Proso Millet (Panicum miliaceum). (Jha *et al*, 2013)

Millets are diverse in its appearance, plant, grain type, maturity and morphological features. And they categorised in two types such as Major millets and Minor millets. Major millets are Pearl millet (Pennisetum glaucum) and Foxtail millet (Setaria italic) which is most widely used for human consumption (Yang *et al*, 2012). Proso millet or white millet (Panicum miliaceum) and Finger millet (Eleusine coracana). Minor millets include Barnyard millet (Echinochloa spp.), Kodo millet (Paspalum scrobiculatum), Little millet (Panicum sumatrense), and Sorghum (Sorghum spp.) (Adekunle *et al*, 2012) .

D. Health benefits

Millets form a great source of magnesium by which it aids in reducing the risk of increased blood pressure and heart strokes and most importantly Atherosclerosis. They also contain potassium which keeps blood pressure low since it serves as a vasodilator. The high amount of fiber present in millet helps to reduce the HDL levels (High Density Lipids). Further researches have shown that the grain sorghum can be used/ consumed in order to manage the human cholesterol levels (Carr *et al*, 2005).

The incidence of major metabolic disorders such as Diabetes mellitus, Cardiovascular disease, Obesity have also increased among the rural population due to erratic changes in the consumption/ dietary pattern and life style (Popkin *et al*, 2012).

Banyard millet (*Echinochloa esculenta)* especially the dehulled variety is found effective in lowering the glycemic index among the Type 2 Diabetics (Ugare *et al,* 2011). The aqueous extract of foxtail millet *(Setaria italica)* is hypoglycemic in nature and hence can be prescribed in treatment of Diabetes mellitus (Srieesha *et al*, 2011).

Benefits of Millets Consumption (Bommy *et al,* 2016)

- Millets are alkaline in nature and easily digestible
- People living in the foothills of the Himalayas are known as the "Hunzas" and they are branded for their good health and longevity. In accordance it is also known that their staple food consists of millets.
- Millets prevent constipation as it rich in fiber and also known to line the colon as it rehydrates.
- Millets also act as prebiotics which feed the inner micro-biota and thus balances the ecosystem.
- Millets contain serotonin which calms the mind and mood (Mitsuru *et al*, 1999)
- Millets is a wise carbohydrate option to chose, as it is high fiber and low in simple sugars which eventually has a lower impact on the glycemic index and also reduces the blood sugar levels when compared to rice/ wheat (Kamari and Sumathi, 2002)
- A study conducted by the Nutrion Research reveals that millets lowers the levels of triglycerides and C- reactive protein. And scientists from South Korea and Seol have found millets to be effective in delaying the occurrence of cardiovascular disorders. (Nutrition Research, *April* 2010)
- A team of researchers opines that all varieties of millets have antioxidative activities (Journal of Agricular and Food Chemistry, J*une* 2010)

Millets are free from Gluten thereby can cater to the needs of celiac disease patients and meet their nutritional requirements. Millets are being fond of as there is soaring demand of gluten free foods (Taylor *et al*, 2006; Taylor and Emmambux, 2008; Chandrasekara and Shahidi, 2011).

Fiber plays a major role in preventing breast cancer in women and sorghum is proven to have anti- carcinogenic properties due to the presence of tannins and polyphenols which have the anti- mutagenic and anti- cancerous/ carcinogenic effect. Millets are beneficial to our colon, gut and the entire digestive system as it forms a natural prebiotic when fermented and stimulates the growth of good bacteria (Laminu *et al*, 2011).

And hence treats diarrhoeal infections in children (Lei *et al*, 2006). Millets can treat and cure problems related to malnutrition and various other health problems. They are an alternative source of therapeutic foods. A study also reveals that millets are "food medicine" (Sujatha *et al* , 2017).

Pearl millet is a form of super food for the treatment of cancer, anaemia, Diabetes mellitus, constipation, non-communicable disease and allergies (Saleh *et al*, 2013*)*.

Protein extracts of Pearl millet (*Pennisetum glaucum)* quality to hinder the pathogenic fungi growth such as Rhizoctonia solani, Macrophomina phaseolina and Fusarium oxysporum (Jeyalakshmi *et al*,2003).

E. Role of Millets in Non-Communicable Diseases

a. Millets for the Management of Diabetes mellitus

Millets has been the major part of dietary pattern right from the civilization era . According to a study the incidence of Type2 Diabetes melitus was very low among the population consuming high fiber diet which composed of whole grains and other millets. And it was shocking to know that In the science of Ayurveda Samai (*Echinochloa frumentacea)* and Kodo millet *(Paspalum scrobiculatum)* were used as the mode of treatment in Diabetes (Pandey *et al,*2009; Shastri,2011).

Some of the studies conducted in recent times depicts that the modifications done in the dietary pattern has the ability to manage the blood glucose levels and enhance insulin sensitivity. Such modification can be made by including Samai (*Echinochloa frumentacea)* and Kodo millet *(Paspalum scrobiculatum)* and other such millets (Pandey *et al,*2009; Dutta ,2011).

Millets are one among the high energy foods and can form a channel in preventing drought and hence can play a pivotal role in handling food security. It is also clearly evident that including whole grains in the diet reduces the risk involved in cardiovascular diseases like increased BMI (Body Mass Index) , insulin sensitivity and Diabetes mellitus. Different epidemiologic cohort studies have exhibited that increase in the number of servings of whole grains reduces the occurrence of Type 2 diabetes by 20-30% with respect to the age, gender and BMI (Body Mass Index) (Munter *et al,* 2007; Venn *et al,* 2004; Montonen *et al* 2003) .

In an Indian study it was found that the glycemic index of pearl millet, barley and corn were compared to that of white bread in those individuals free from Diabetes mellitus and was surprising to know that the pearl millet and corn had lower glycemic index (Shukla *et al,* 1991) .

For the diabetic patients in India it was demonstrated that wheat and millet based formulations evinced lower glycemic indices in comparison with rice and its products (Shobana *et al,* 2007*)*

The phytochemicals are held responsible for the anti-diabetic property of kodo millet *(Paspalum scrobiculatum)* . Quercetin , is the main flavonol in this millet that has a lot of medicinal properties like being anti-diabetic . In an in vitro study conducted it was found that Quercetin is capable of decreasing the intestinal glucose absorbtion, block tyrosinase kinase, activate both glucose and glibenclamide induced insulin secretion and protects β-cells from oxidative damage and enhance glucose homeostasis (Piedra *et al,* 2010; Kwon *et al,* 2005).

One of the most important action of Quercetin is in the management of obesity as its evident from the study conducted by (Hagde, *et al,* 2005*)* that it decreases the triacyglycerol level, preventing lipogenesis and lipoprotein lipase , activation of lipase and thereby increasing lipolysis, influence weight loss and reducing oxidative stress.

In an *in vivo* study various beneficial activities of Quercetin was shown and they are;

a) Inhibition of small intestine maltase
b) Increased glucokinase activity and number of pancreatic cells of islets
c) Partial prevention of degeneration of β-cells
d) Reduce the symptoms of diabetes and liver injury
e) Enhance insulin sensitivity and it is also studied that foods rich in quercetin are more effective in managing diabetes than the pure form of quercetin. (Kiran *et al,* 2013; Youl *et al,* 2010; Jain *et al,* 2010)

The incidence of diabetes is reportedly reduced among the millet consuming population. Millet phenolics like alpha-glucosidase, pancreatic amylase reduce the postprandial blood glucose level through the partial inhibition of enzymatic hydrolysis of the complex carbohydrates (Shobana *et al,* 2009). Millets are also capable of preventing diabetes induced cataract problems by the prevention of sorbital accumulation where aldolase reductase acts as an inhibitor (Chethan *et al* , 2008).

b. Millets for the Management of Cardiovascular Disease

Millets are a great source of magnesium by which it prevents the effects of migrane and heart attack. Millets are known to reduce the cholesterol levels as phyto-chemicals containing phytic acid are present in them . Finger millet may prevent cardiovascular disease in hyperlipidemic rats by decreasing the plasma triglycerides (Lee *et al*, 2010).

Potassium content in millets aids in reducing the risk of hypertension, since it serves as a vasodilator. They also have the ability to prevent cancers and other cardiovascular disorders, as they ar capable of converting plant lignans into animal lignans. Fiber content is equally important as lowers the LDL and increases the HDL levels. Also there are enough evidence to show that sorghum and proso millet may bring down the plasma triglyceride levels.

Antioxidants such as lignan, phytonutrients keeps the heart related problems at bay. Millets such as pearl, finger and banyard have these properties. (Lee *et al*,2010). Especially the proso millet increases the plasma adiponectin and HDL levels better (Park *et al,* 2008).

c. Impact of Millets in Celiac Disease

Gluten intolerance or celiac disease is a genetically inherited disorder which is an immune mediated enteropathy. Millet is a gluten-free food which makes it an amazing option for gluten sensitive patients (Saleh *et al*, 2013)

d. Millets as Antinutrients

Millets also contain several phenolic acids, tannins and phytate which at times act as ‘antinutrients’. But they reduce the occurrence of colon and breast cancer in animals and also proven that millet phenolics may be effective in the prevention of cancers (Chandrasekara *et al*, 2011)

In recent times polyphenols are known to increase the life span as it maintains all the body functions throughout the life and hence termed as “life span essential” (Chandrasekara *et al,* 2010). Millet polyphenols are a combination of cinnamic acid derivatives and benzoic acid which are known to conduct enzyme inhibitory and anti-carcinogenic activity (Devi *et al,* 2003)

Cereals contain major polyphenols such as phenolics acid and tannins and trace amount of flavonoids (Muralikrishna *et al,* 2002). Millets in general have anti-oestrogenic, anti-mutagenic,anti-carcinogenic, anti viral, anti inflammatory and platelet accumulation inhibitory activity which by nature prevents the occurrence of ailments (Fergusson, 2001).

F. Antioxidants in Millets

In general antioxidants have a health promoting benfits. Antioxidants are known to prevent free radicals in our body and thus prevent cancers. Some of the antioxidants are phenols, tannins, phytosterols, anthocyanins (Awika and Rooney, 2004) And millets specifically soghum has increased levels of phenols, condensed tanins and flavonoids. (Subba Rao and Muralikrishna, 2002)

Sorghum millet is a potential detoxifyer as it can combine all the toxins. Pearl millet, sorghum and finger millet is considered heart healthy as it decreases the occurance of cardiovascular disorders and also has an impact over reducing the cholesterol levels. (Rao, 2003)

Antioxidants from plant source like flavonoids and other such phenolics compounds have various biological activities including that of antioxidants. Plant based phytochemicals have many beneficiary health effects since they confront oxidative stress and thus strikes a balance between oxidants and antioxidants (Scalbert *et al,* 2005*)*

Bioactive elements are considered to be added nutritional compounds actually present in minor quantities in foods , but are not necessary but useful to health (Kris *et al,* 2002)

Phytochemistry of Millets

The millet Banyard (*Echinochloa esculenta)* is packed with amino acids like lysine (0.046-0.047%), aspartic acid (0.522%) , methoinine, L-ornithine, glutamic , arginine, alanine, serine, glycine, proline, threonine, valine , tyrosine, leucine, phenylalanine, isoleucine and L-hydroxyproline. It also contains saturated fatty acids like palmitic acid(17.1%), caprylic acid (2.1%), stearic acid(6.1%). The overall total saturated fatty acid content is (24.2-26.0%). The unsaturated fatty acid content comprises of oleic acid(29.5%), linoleic acid(46.9%)and linolenic acid (1.0%) (Gupta *et al*, 2005).

The main flavonoid present in kodo millet is Quercetin. The major five phenolics acids found in this millet are vanillic acid, syringic acid, cis ferulic acid, p-hydroxy benzoic and melitolic acid. The total gallotannin form of phenols present are 1.20mg/g. during the assay 0.856% of a clear yellow fatty oil was obtained which consists of esters of four major fatty acids and they are Oleic acid, Stearic acid, Palmitic acid and Linoleic acid . Among all , the saturated form of fatty acids were more amounting to 57%. And they comprised of 37.5% of stearic acid , 19.5% of palmitic acid. In the unsaturated acids oleic acid was maximum (40.7%) and lenoleic acid was very less (1.57%). The phospholipid content of the grain was 0.24% and consisted of four bands of cephalins , two bands of lecithin and one band of galacto lipid (Kiran *et al*, 2013)

Also from a study done by (Sanjay *et al*, 2010*)* it is understood that vanillic acid has the potential to prevent diabetic neuropathy and this is possible by blocking the methyl glyoxal-mediated intracellular glycation system. Cell viability is improved and apoptosis of cells is reducedby both syringic and vanillic acid .

Nutrients such as minerals, vitamins essential fatty acids and fiber are believed to be responsible for promotion of health , but it becomes more beneficial when the bioactive compounds react with nutrients like oligosaccharides, lipids, antioxidants (flavonoids, phenolics acids, avenanthramide), hormone stimulating compounds like (lignans , phytosterols) and anti nutrients like(tannins, phytic acid) (Edge, 2005)

It has been documented that millets serve as functional food and as nutraceuticals. Obesity and Diabetes have become a global endemic and consumption of foods rich in complex carbohydrates and high fiber helps to treat such health issues (Shobana *et al*, 2007).

In the medical field ,more of whole grain based formulations are being developed since it is an excellent source of phytochemicals and dietary fiber (Jones *et al*, 2010)

A study carried out by (Kumari *et al*, 2002) states that finger millet (*Eleusine coracana)* reacts to lower glycemic level and this is because of the presence of anti-nutrients which decrease digestion and absorption of starch. There is phenolics and dietary fiber present in the seed coat of finger millet (Chetan *et al*, 2004) and they play the role of inhibiting postprandial rise in blood glucose levels by hampering the enzyme activity (amylase, α-glucosidase) which is required for the complex carbohydrates hydrolysis (Shobana *et al*, 2009).

Polyphenols inhibit the action of digestive enzymes such as glucosidase, pepsin, amylase, lipases and trypsin (Rohn *et al*, 2002)

A study done by (Banerjee *et al*, 2012) expresses that phenolics and flavonoid content of finger millet inhibits the oxidation of microbial membranes and enzymes which affects the multiplication of bacterial cells like Escherichia coli, Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus, Streptococcus pyogenes, Serratiamarcescens, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia and Yersinia enterocolitica.

A study done by (Xu *et al*, 2011) evinces that millet polyphenols are antibacterial and antifungal in nature. Among the phenolics acids ferulic, caffeic, protocatechuic and p-coumaric shows major anti-fungal effect (Dragland *et al*, 2003).

Millet flavones like kaempferol, quercetin, apigenin and luteolin are also associated to antiproliferative activities (Manthey *et al*, 2002).

G. Classification of Dietary Fibers

Fibers are often classified on the basis of their source (Plant, animal, isolated, synthetic),but can be differentiated on the basis of chemical, physical or physiological aspects (Englyst *et al*, 2005)

i. Chemical (Polymer length and types of linkages).

Oligosaccharides are either

a.) maltodextrins (α-glucans), primarily resulting from the hydrolysis of starch

b.) non - α-glucan like raffinose and stachyose

Polysaccharides are again divided into starch (α-1,4 and 1,6 glucans) and non-starch which mainly consists of cellulose, hemicelluloses and pectin along with plant gums, mucilages and hydrocolloids (Roberfroid et al, 2007)

ii. *Physical (Solubility and Viscosity)*

Fibers are generally classified on the basis of solubility and this is based on its solubility in hot aqueous buffer solution .But the solubility of the dietary fiber structure can be found in aqueous enzyme slurries. (Cho *et al*,1997).

iii. *Physiological (Rate of Digestion & Fermentation)*

Factors involved in the rate of carbohydrate digestion involves the rate at which CHO leaves the stomach and gets available for absorbtion and the diffusion of released sugar (Englyst; Liu *et al*, 2007). Hence , the rate at which CHO leaves the food matrix and the ability of amylase to act on it decides the blood glucose levels (Wong *et al*, 2006)

Based on the digestion rate , CHO is classified as rapidly / slowly digested and even as resistant. The resistant CHO includes plant cell wall polysaccharide, gums, fructans, resistant maltodextrins and resistant starches. And these resistant starches reaches the large intestine to render prebiotic effects through fermentation (Macfarlane *et al*, 2006).

According to the studies conducted by (O'Keefe,2016; Peters *et al*,2003*;* Mao *et al*, 2017) prebiotics such as dietary fiber, have been evaluated due to their potential to modulate intestinal microbiota. They may be able to improve the selection of protective bacteria like Lactobacillus and Enterococcus and thus help to prevent cancer.

(Ohira *et al*,2017) suggests that that the risk of colorectal cancer is increased by the consumption of processed and unprocessed meat, but has reduced over the consumption of fiber.

DIETARY FIBER

Dietary fiber and whole grains form an exclusive composition of bioactive elements which involves resistant starches, minerals, vitamins ,antioxidants and phytochemicals and their impact on promoting health positively has been in limelight for the past few years. There are several epidemiological studies stating that, not only management of obesity can be done through increased consumption of high fiber and whole grains (Tucker *et al,* 2009) but also Type 2 diabetes(Meyer *et al,*2000*),* cancer (Park *et al*, 2009) and Cardiovascular disorders (Streppel *et al,* 2008).

The Food and Drug Administration has approved two health claims in regard with the dietary fiber and one those states that "Increased fiber consumption and decreased fat consumption according can decrease the risk of some types cancers" (FDA, 2008)

The synergetic effects of phytochemicals have the potential to increase the nutritional and digestive properties and this is to be supposedly the reason for the dietary fiber to effectively manage diabetes and obesity (Weickert *et al,*2008) and to reduce the risk of cardio vascular diseases (Liu *et al,* 1999) and also to bring down the rate of certain types of cancers (Chavan *et al,* 2001;Terry *et al,* 2001).

It was amazing to know that the major health benefits of beta glucan derives from its potential over lipid metabolism and postprandial glucose metabolism and also there are several supporting the positive relationship between beta glucan and the cholesterol levels. A recent study conducted by (Davidson *et al,* 1999) reveals that 5g of beta glucan can remarkably reduce the serum total and LDL cholesterol level among both normal (Naumann *et al,*2006*)* and hypercholesterolemic subjects (Theuwissen *et al*, 2007).

In contradiction, also there are studies proving that only 3.6g of beta glucan is sufficient to render the same health beneficial effects. And a similar relationship exists between beta glucan and postprandial glucose and response to insulin among both diabetic and healthy individuals (Biorklund *et al,*2005).

Most of studies opines that the credit of reducing the serum cholesterol levels and postprandial glucose metabolism goes to the viscosity of beta glucan in the Gastrointestinal tract. And this process of gellation reduces the absorption of bile acid but increases its excretion and also improves the intestinal viscosity. Due the above mentioned mechanism , synthesis of hepatic cholesterol increase and there leading to more production of bile acid (Lia *et al,* 1995*).* At the same time this increased level of viscosity may slower the process of glucose absorption and insulin levels (Nazare *et al*, 2009)

(Steinberg *et al,* 2009*;* Kaczmarczyk *et al,* 2004) suggests that Dietary fiber influences the expression of genes that regulate lipid and carbohydrate metabolism. It has been demonstrated that resistant starch, fructans, inulin and β-glucans affect the phosphorylation of AMP-activated protein kinase (AMPK). Which is a key enzyme involved in energy exchange.

(Drew *et al,*2004) highlighted in a study that higher concentrations of blood HDL protect against cardio vascular diseases. ApoAI activates AMPK in the arterial endothelia and increases the phosphorylation and activity of nitrogen oxide synthase.

Consuming dietary fiber renders many beneficial nutrition and health benefits. Dietary fibers have been proven to reduce the risk of cardiovascular diseases, diabetes mellitus, certain type of cancers,, and improves the short and long-term memory functions. The (WHO,2007) recommends a dietary fiber intake of at least 25g per day.

The FDA have adopted a recommendation of 3g of β-glucan per day as having a nutritional affect, which is a part of recommended 30-35g of dietary fibre per day as advised by the American Association of Dieticians (FDA 1997).

H. Beta-Glucan and Its Properties

a. Beta –Glucan Definition

Glucans are nothing but the polymers of glucose which again classified as α or β- linked based on their interchain linkage (Barsanti *et al,* 2011). They are a group of heterogeneous non-starch polysaccharides mde of D-glucose monomers linked by β- glycosidic bonds (Zekovic *et al,* 2005). The source of beta-glucan and the method by which it is isolated play a major role in framing the molecular structure (macro) (Mc Intosh *et al,*2005). The most simplest structures of beta-glucan is found in the non-lignified walls of cereal grains containing β-(1,3)(1,4)-D-glucans (Wood *et al,*2007).

b. Physiological effect of β-Glucan

The time required for the carbohydrate to digest depends on various factors also including the phase at which the carbohydrate leaves the stomach and available for the absorption and the breakdown of resulting sugars takes place simultaneously from the food.

Hence the time taken for the carbohydrate to separate from the food matrix and potential of amylase to react with the carbohydrate is the major determining factor of glucose absorption and blood glucose levels (Wong *et al,* 2006*).*

Carbohydrate classification such rapidly, slowly digested or even as resistant is done on the basis of its digestion capacity. Resistant carbohydrates involves plant cell wall polysaccharides, fructans, gums, resistant maltodextrins, and resistant starches. Some of the insoluble fibers such as (lignins, cellulose, hemicelluloses) are resistant to fermentation but the soluble fibers like (pectins, gums, mucilages and some hemicelluloses) are fermented by colonic microflora to the maximum (Gibson *et al,* 2004).

c. Sources and isolation (Oat β-glucan)

A viscous polysaccharide made up of units of the monosaccharides,D-glucose is derived from oat kernels. Around 20-30% of the total weight comprises of the hulls in the oat kernels of common cultivar types. The oats kernel approximately contain 85% of insoluble dietary fiber in its unprocessed state. Naked cultivar varieties are exempted due to negligible amount of hull content (5%). Hulls can be further processed to obtain oat hull fiber which is high in the insoluble dietary fiber content (>90%) (Cho,2001)

In the remaining edible part, the groat, the total content of dietary fiber is generally 6-9%, half of it being insoluble fiber, found mainly in the tissues outside the aleurone layer. The principle component of oat soluble fiber is the linear polysaccharide (1,3),(1,4)-β-D-glucan, commonly known as β-glucan. It is located in the endosperm cell walls, and they are thickest located adjacent to the aleurone layer in the sub-aleurone layer. The size of the endosperm cells, thickness of the cell walls throughout the groat and thereby the distribution of β-glucan vary widely according to the different cultivar varities (Fucher and Muller, 1993).

Studies performed by (Malkki *et al*,2001*;* Paton *et al,* 1995) reveals that the oat bran contains about 55-90 g/ Kg , oat groat contains 35-50g/Kg and oat gum contains 600-800g/Kg of beta-glucan.

According to the study done by (Cui and Wood, 2000) , the relative amounts of oligosaccharides released from β-glucans through hydrolysis with (1,3),(1,4)- β-D-glucan-4-glucanohydrolase (lichenase), which exclusively cleaves the (1,4)-linkage of a 3-O-substituted β-D-glucopyranosyl residue consists of a fingerprint of the structure of the β-glucans. The molar ration of tri/tetra oligosaccharides 2.1:2.4 is specific to/ characteristic for oats.

In an extractive study (Asif *et al,* 2010) performed acid, alkaline and enzymatic extraction and purification of oat β-glucan. They observed that enzymatic extraction was the best , since it produced the highest yield and also removed excessive starch, fat and pentosans in the extraction process of β-D-glucan gum, along with a good amount of minerals by not affecting the physiochemical properties to a large extent.

I. Potential Health Benefits of Beta glucan

β-glucan, based on its structure and source has commendable effect on cardiovascular disorders due to its antibacterial, antitumoral and other positive physiological effects. The active component contained in certain foods has positive effect on health and such foods called functional foods, where as β-glucan are also an active component. (Passarelli *et al,2011*) highlights that natural products that may prevent or treat various diseases have been identified, which also includes β-glucans.

From a study conducted by (Charalampopouloset *et al*,2002; Demi 2005; Holtekjolen *et al* , 2006) it is evident that major site for most of the glucans is the internal and sub aleurone endosperm cell walls .

Based on the study done by (American Association of Ceral Chemists, 2001; Andlauer & Furst 2002;Charalampopoulos *et al*,2002) we can understand that fiber is the edible or digestible component of plants which cannot be hydrolyzed by enzymes but can be metabolized to produce volatile fatty acids. Among them there are also water soluble fiber which are non-starch polysaccharide such as Beta-glucan and Arrabinoxylan.

Beta glucans are widely used in the field of medicine for the treatment of non-communicable diseases like hypercholesterolemia, diabetes, cancer and AIDS; they also act as immune modulators (Heike *et al*, 2014)

(Dhingra *et al*,2012) has proven that Like any other polysaccharides and dietary fibers, β-glucan mechanism of action includes reduction in nutrient absorption and improving viscosity of intestinal contents. β-glucan can be a potential source for the fermentation by the microbes in the small intestines and therefore can also produce prebiotic effect.

a. Effect of Beta glucan in heart health

The U.S. Food and Drug Administration (FDA) have approved that high amount of beta glucan enhancement the heart healthy and decrease the risk of heart attack through its effect on concentration of cholesterol. B-glucans are characterized by increase the viscosity of aqueous solutions, especially in the upper gastrointestinal tract, leading to an increased binding of bile acids and their subsequent excretion which in turn decrease plasma cholesterol levels (Varma *et al*, 2016*).* There is inversely correlation between bile acid synthesis and cholesterol level (Myriam *et al,* 2018).

b. Effect on Immunity

Several studies indicated that beta glucan could have some positive effects on the improvement immune system. It binds to a specific receptor site on the surface of all immune cells with CR3 receptors. This binding improves the effectiveness of these immune cells to attack any foreign substances such as, viruses, bacteria, fungi, parasites (Mendonca *et al*, 2017*)*

Also aids in chemoprevention, Beta glucan act as anti-cancer agent, primary reason mediated by the reduction of reactive oxygen species and this protect DNA from damage, whereas secondary reason it inhibits the growth and further transformation of cancer cells (Vahid *et al*, 2017*).*

(Novak *et al*,2008) states that the immune modulation effects of beta glucan are well established during the development of immune reactions as in certain medical conditions like chronic fatigue syndrome, physical and emotional stress. And that this effect results from glucans is proved to be macrophage stimulants, and that macrophages are key factor to immunity.

According to (Barros *et al,*2016) beta glucans are used for the treatment of various types of diseases which are associated with immune system infections. And beta glucans are also used in industries as a food additive in several products.

β-glucans are also used to improve the efficiency of the immune system. The immune modulatory properties primarily depend on the source they have been extracted from, studies carried out on animals and humans confirmed that the oral administration of yeast β-glucans had a better effect on the immune system than cereal/ grain derived β-glucans (Novak *et al,2008;* Vetvickova *et al,* 2016).

c. Effect of Beta Glucan in Dyslipidemia

Individuals with metabolic syndrome often present with atherogenic dyslipidemia, characterized by elevated concentrations of triacylglycerols and low levels of HDL cholesterol in blood (Torpy *et al,* 2006)

This lipid profile presents an individual with a high risk for cardiovascular disease. Soluble fibers have the most reported beneficial effects on cholesterol metabolism. In a meta-analysis, soluble fibers pectin, psyllium, oat bran, and guar gum were all proven to be equally effective in reducing plasma total and LDL cholesterollevels (Brown *et al,* 1999).

When included with in a low saturated fat and cholesterol diet, soluble fibers lowered LDL cholesterol concentrations by 5–10% in hypercholesterolemic and diabetic patients (Sierra *et al,* 2002)

According to the studies conducted by (Ho *et al,* 2016; Anderson *et al,* 2009; Horn *et al,* 2008; Vetvicka *et al,* 2009) it has been demonstrated that β-glucans reduce the levels of a non High Density Lipoprotein – Cholesterol (non HDL-C) fraction that containsLDL-C without HDL-C or triglyceride levels.

d. Cholesterol reducing Mechanism

Based on the study conducted by (Ellegard *et al*,2007*;*Theuwissen and Mensink ,2008) the cholesterol reduction is evidently a sum of several effects. Although it is a commonly accepted concept that the main mechanism for β-glucan’s cholesterol- lowering effect is assumed to be dependent on its ability to entrap whole micelles containing bile acid in the intestinal contents mainly due to its viscosity and excluding them from the required interaction with the luminal membrane transporters on the intestinal epithelium , and hence decreasing the absorption or re-absorption of fats, which comprises of cholesterol and bile acid and thereby increasing its fecal output.

e. Effect of Beta Glucan in Insulin resistance

Though associated with hyperglycemia and type 2 diabetes or not . It is a major element of metabolic ssyndrome (Xu *et al*,2010). β-glucan, pyllium and guar gum and other such soluble fibers reduce the postprandial glucose and enhances the insulin sensitivity among diabetic and non-diabetic individuals (Alminger *et al*,2008).

β-glucan also contributes to the glycemic control and other aspects that influence are interaction, food form and molecular weight. When compared other fibers , a small amount of β-glucan is enough to decrease the postprandial glucose and insulin response in healthy subjects (Maki *et al*,2007) and type 2 diabetic patients (Tapola *et al*,2005).

Studies conducted by (Brennan *et al*,2003 *;*Li *et al*,2003) reveals that in general, food high in soluble dietary fiber have been shown to have a positive effect on reducing hyperglycemia and hyperinsulinaemia, in relation to the control of diabetes

(DeVries *et al*,2001) suggests that the most extensively documented nutritional benefit of β – glucan in foods is the flattening of postprandial blood glucose and insulin rises which can be performed by the oats β – glucan. And also FDA has acknowledged nutritional claims that the use of dietary fibers reduces the glycemic and cholesterol responses of individuals. An intake of 20-40g of dietary fiber per day is regarded beneficiary.

There is a supporting done by (Jenkins *et al*,2002) indicating that 1g of β –glucan per 50g of carbohydrate consumed can reduce the glycemic index of food by 4 units.

f. Effects of Oat β-glucan in Gastrointestinal tract

A study conducted by (Malkki and Virtanen,2001) reveals that both the kind of fibers – soluble and insoluble have gastrointestinal effect. Soluble fiber can greatly swell and also has the ability to bind water along with being a substrate in colon fermentations. The insoluble fiber has the bulking effect and thus they have a positive effect over the gastrointestinal tract.

A study done (Ulmius *et al*,2012) opines that oat β-glucan has a smaller hydrodynamic size, and its aggregates seems to decreased or disrupted for which low pH could be one of the possible reasons as there was no change found when the enzyme pepsin was absent in the stomach.

After the digestion in small intestine, the β-glucan molar mass increases irrespective of whether bile acids or enzymes are present or not, and this means that no binding of β-glucan with bile acids or pepsin/pancreatin was noted, instead the rise in aggregate size could be a result of the normalized pH (6.8). The reformed aggregates resulted in a range of Mol.Wg.(200-700x 106g/mol for oat bran β-glucan, 10-100 x 106g/mol for pure β-glucans), indicating that β-glucan aggregation could lead to aggregates with varied sizes (Ulmius *et al*,2012).

The behavior of β-glucan during gastrointestinal digestion indicates that different fiber fractions have different optimal acidification levels where the polymers are released and viscosity increases. Beyond these levels, polymers break down and lose their viscous effects (Dikeman and Fahey, 2006).

(Ulmius *et al*,2011) have confirmed that β-glucan from pure fractions and oat bran behaves differently in the gastrointestinal tract and may have different mechanisms of action.

A study conducted by (Malkki *et al*,2001) reveals that in the stomach and small intestine, oat-soluble fiber β – glucan acts primarily by increasing the viscosity of the gastric and intestinal content, this action is mediated via neurohormonal systems involving both endocrinal and gastrointestinal hormones. Oligosaccharides produced from β – glucan have been demonstrated to act as selective factors which favours the growth of some beneficial probiotic strains.

(Reimer *et al*,2000) suggests that the potential reduction of cholesterol is considered to be the result from effects manifest in the upper gastrointestinal tract. And this action may be due to ability of cereal fiber to form a gel-like network and alter gastrointestinal viscosity.

g. Effect of Beta Glucan in Blood pressure

Hypertension is another dangerous non-communicable disease which becomes the reason for elevated risk of cardiovascular disorder ,diabetes mellitus , stroke and renal diseases (Chobanian *et al*,2003) . The main causative agent is obesity and high stress levels which can be prevented by following a fiber rich diet.

h. Prevention of cancer

Antitumor and anticancer effect of β-glucan is not just being macrophage that attack tumor cells and destroy them, but also modulate the lymphocyte, neutrophil and natural killer (NK) cells activity and other components of the innate immune system (Hong *et al*,2004). Several studies on antitumor properties of β-glucan are carried out with various types of mushrooms and fungi, but all contained (1, 3)- β-glucan (Demir *et al*,2007; Liu *et al*,2009;Chen *et al*,2011).

(Murphy *et al*,2004) after examining the independent and combined effects of short-term exercise and oat β-glucan consumption (raw oat β-glucan was fed in the drinking water) on the metastatic spread of injected tumor cells (B16 melanoma cells through intravenous injection), and macrophage antitumor cytotoxicity, proved that the metastatic spread of injected B16 melanoma cells was similar in all groups (group mice exercise plus water, group mice exercise plus oat β-glucan, and group mice control plus oat β-glucan) and had a marked difference when compared to the group control plus water.

(Khawaja *et al*,2017) opines that among the benefits of beta glucan , its role in treating cancers is commendable as radiotherapy is the chief method for treating vigorous tumours, and while in the treating process, beta glucan represents as a guard from damage, and also helps in the improving blood cells formation. Beta glucan is used in treating various types of cancers like pulmonary malignancy, prostrate cancer (Li *et al*,2017) and breast cancer (Taek *et al*,2013).

i. Antimicrobial and immune effects of oat β-glucan

As much as fungi-derived β-glucans may have stimulatory effects on the immune system, leading to resistance against viral, bacterial, parasitic, and fungal pathogens, the cereal derived β-glucans have also been ascribed to have immune-stimulating properties. It was reported that natural β-glucan administration (intravenously or intramuscularly or taken orally) helps in the elimination of bacteria by increasing bacterial clearance, increasing bactericidal activity, increasing modulation of cytokine production, and increasing the number of monocytes and neutrophils, thereby resulting in an antibiotic potential (Liang *et al*,1998; Kaiser *et al*, 2002)

Some scientists reported that oat β-glucan taken orally alone or in combination with sucrose has beneficial effects on susceptibility to HSV-1 respiratory infection and macrophage antiviral resistance following stressful exercise (Davids 2004; Nieman 2008 ; Murphy *et al,* 2008 *).* Oat β-glucans increase the activity of transcription factors in intestinal leukocytes and enterocytes of β-glucan treated mice (Volman *et al* ,2010).

j. Beta Glucan in Functional Foods

Fiber fortification is a great source of high fiber intake . And can also be incorporated in the available functional foods, where inulin is the major component (Aykan *et al*,2008). And β-glucan is widely used as a functional ingredient (Lee *et al*,2009).

(FDA,1997) highlights that Oat products are a widely studied dietary source of the cholesterol lowering soluble fiber β-glucan. There is a scientific evidence supporting that the consumption of this plant food specifically can decrease the total and low density lipoprotein (LDL) cholesterol level in patients, and eventually as a result it reduces the risk of coronary heart disease (CHD).

Oat bran is a dietary fiber and β–glucan enriched oat fraction that can be used in products aiming towards improved nutritional status. Long established oat bran products have β –glucan content around 55-90g, but the oat bran total dietary fiber content is remarkably higher than β –glucan content. Oat β –glucan has in the native state a chain length of about 20,000 glucosidyl units, which gives molecular weights up to 3 million Daltons (Wood *et al*,1991).

k. Antioxidant effect of beta-glucan

The valuable physiological and nutritional attritubes of oat by β-glucans and other dietary fiber components, high tocopherol and natural antioxidant level have generated a high demand for oats in human nutrition as told by (Zwer,2004). (Zielinski *et al*,2001*)* observed that vitamin E activity is contributed by the tocopherols and tocotrienols, which together form tocols. Alphatocopherol is the principle antioxidant component in crude oat unaltered when the lipid is refined. Seperated fatty acids (FA) also have consequence on human health. There many supporting studies that estimate the beneficial effects of polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA), proved (Chillard *et al*,*2000 and* Gebauera *et al*,2005).

The content of β-glucans of oat grain samples ranged 2.7g to 3.5g where average content of β-glucan for naked oat varieties determined 4.99 g100g-1 and 5.07 g100g-1 (Brunava *et al*,2014). In the existing literature, β-glucan content for oat reported 2g/100g to 8g/100g of oat groats and is seemingly influenced by genetic and environmental factors, mentioned by (Welch *et al*,1995*)*.

J. Physio-Chemical Properties of Beta-Glucan

a. Determination of Beta-Glucan

(Tosh *et al.*, 2003; Du *et al.*, 2014) obsereved that different extraction methodologies and techniques .The cereals containing β-glucan is present in walls of the endospermic region of cells with lipid and protein matrix, so the extraction process is not simple. When extracted β-glucan analyzed for different quality attributes needs optimum conditions and purity of β-glucan. Different methods and techniques have been developed for purification and isolation and to get potential bioactive compound (β-glucan) various critical points should be considered. It has been found that the molecular weight of the isolated β-glucan is directly proportional to the methodology applied for extraction. The extraction techniques from cereals comprises of three basic steps i.e to inactivate endogenous enzyme, isolation of β-glucan and finally to precipitate the extracted β-glucan. The enzyme inactivation includes refluxing above 60^{o} C with aqueous ethanol (Charles and Cleary, 2005*)*

b. Sensory attributes of β-Glucan

β-Glucan is a fibrous polysaccharide and good source of soluble dietary fiber having multiple functional and medicinal properties.. The in vitro antioxidant activity of cake increased from 18.23 to 22.58% with increase in β-glucan level from 2.5 to 7.5%. The volume index of cake was improved with β-glucan addition up to 5.0% and decreased with further addition to 7.5%.

(Seema Sharma *et al*,2018) found that the TPA characteristics of the cake improved significantly with β-glucan addition where as firmness decreased with increase in β-glucan content. Panelists rated highly cake fortified with β-glucan levels up to 5.0%. The decrease in sensory score at higher β-glucan level could be attributed to the increase in gumminess, cohesiveness and decrease in resilience score. Overall, the addition of upto 5.0% β-glucan extract in wheat flour cake improved its physicochemical, textural, sensory and in vitro antioxidant characteristics.

c. β-Glucan content of various cereals

Mixed-linked (1 → 3), (1→ 4)-β-d-glucan contents of 14 selected cereal grains such as barley (Hordeum vulgaria), beans, canary seed (Tropaeolum peregrinum), corn/maize (Zea mays), flax, lentil (Lens culinaris), millet (Panicum miliaceum), oat (Avena sativa), peas, rice, rye (Secale cereale), spelt (Triticum spelta), spring wheat and winter wheat, were determined quantitatively using enzymatic methods.

By using pure β-d-glucanase and β-d-glucosidase in the experiments, (1 → 3), (1→ 4)-glycosidic bonds of linear polysaccharides found in cell-wall endosperm of plant seeds were hydrolyzed and the resulting β-d-glucans were determined by using glucose oxidase/peroxidase solution and measuring the absorbances at 510 nm in a UV-spectrophotometer. The nutrient mineral contents of the 14 selected cereal grains were studied.

Some macronutrients such as K, Ca, Mg, N, P and S, and some micronutrients, such as Zn, Cu, Fe, Mn, Mo, and B, were analyzed by using atomic absorption spectrometric (AAS) methods. A flame photometer was used for determination of potassium. After oven drying of the samples, P was determined by a colorimetric method. (Demirbas, 2005)

d. Digestibility of Beta-Glucan

(Curti *et al*, 2017) suggests that minor grains such as sorghum, millet, quinoa and amaranth can be alternatives to wheat and corn as ingredients for whole grain and gluten-free products. In this study, influences of starch structures and other grain constituents on physicochemical properties and starch digestibility of whole flours made from these grains were investigated. Chemical composition and amino acid profile of whole grain flours were compared.

Physicochemical properties and enzymatic hydrolysis of starch varied with grain type. Starch molecular features were the major factors affecting thermal, pasting and enzymatic hydrolysis properties. Additional effects of non-starch constituents on whole grain characteristics are dependent on botanical source.

Starches were classified into two groups according to their amylopectin branch chain-length: (i) quinoa, amaranth, wheat (shorter chains); and (ii) sorghum, millet, corn (longer chains). Such amylopectin features and amylose content contributed to the differences in thermal and pasting properties as well as starch digestibility of the flours.

e. Comparison of dietary components in cereals & legumes

The content of total fiber as well as certain fiber fractions was determined in cereals, cereal products, and cooked legumes. The content of total fiber in cooked cereals and cereal products ranged from 2.5 to 20.8 g/100 g, and in cooked legumes from 14.0 to 24.5 g/100 g (on dry matter basis).

Distribution of analysed fiber fractions and their quantities differed significantly depending on food groups. Fructans and arabinoxylans were the most significant fiber fractions in rye flakes, and β-glucan in oat flakes, cellulose and resistant starch were present in significant amounts in peas and kidney beans. The same foods were the best sources of cellulose (4.98 and 3.56 g/serving) and resistant starch (3.90 and 2.83 g/serving). (Vesna *et al*, 2013)

f. Degrading enzymes from native and malted finger millet

(Nirmala *et al*, 2000) opines that a recently released hybrid ragi, Indaf-15 was germinated up to 96 h at 25°C and the sprouts, drawn at 24 h intervals, were dried, devegetated, powdered and evaluated for malting loss, reducing sugar, free sugar profile, starch content, dietary fiber and an array of carbohydrate-degrading enzymes. Malting loss was maximum (32.5%) at 96 h. The total reducing sugar content increased from 1.44 to 8.36%, whereas the total carbohydrate content decreased from 81 to 58% at 96 h of germination.

Analysis of 70% alcohol-soluble sugars revealed glucose, fructose and sucrose in different proportions with respect to germination time. Maltose and maltotriose were detected after 48 and 72 h, respectively. There was a linear decrease in starch content (from 65 to 43%). Activities of amylase and pullulanase were maximum at 72 h whereas those of α–d-glucosidase and 1,3–β–d-glucanase, were maximum at 48 h. Xylanase activity was maximum at 96 h with a concomitant decrease in arabinose to xylose ratio from 1:1 to 1:0.38 in the dietary fiber.

α-Galactosidase activity was negligible, which is in tune with a very small amount of raffinose series oligosaccharides. The above results indicated that Indaf-15 is a potential variety for malting purposes as it develops high levels of amylases during germination, and its malt form is a rich source of reducing sugar.

g. Effect of fermentation on β-Glucan

Oat bran was fermented by filamentous fungi to improve the extractability of β-glucan. Box-Behnken experimental design and response surface methodology were used to obtain the maximum extractability of β-glucan. Inoculum volume, fermentation temperature, and time were evaluated as three variables.

Filamentous fungi fermentation increased extractability of β-glucan in oat bran for 3-fold. The molecular weight and viscosity of the extracted β-glucan decreased after fermentation.The increased extractability compensated for the reduced molecular weight in producing viscous solution. The molecular structure and cellotriosyl/cellotetraosyl ratio were not affected by filamentous fungi fermentation. (JiaWu *et al*,2018)

h. Water-solubility of β-1,3-glucan

(Zhuabc *et al,* 2019) conducted a study that involved a preparation of water-soluble β-1,3-glucan (WSG) by hydrolysis of curdlan with H2O2. The hydrolysis conditions were optimised as follows: reaction time of 40 min, H2O2 concentration of 2%, temperature of 85 °C and NaOH concentration of 2 mol/l. Under these reaction conditions, WSG yields of up to 83% could be achieved. High-performance liquid chromatographic analysis of WSG revealed a molecular weight of 5268 Da.

Fourier-transform infrared spectroscopy confirmed the polysaccharide structure of the WS products. HPLC analysis of the complete hydrolysate of WSG indicated that WSG product consisted of glucose. Taken together, the results demonstrate that preparation of WSG by hydrolysis of curdlan with H2O2 is a promising approach with practical significance.

i. Reactive oxygen species responsible for beta-glucan degradation

The presence of iron(II) in beta-glucan in solution causes the formation of hydroxyl radical, which further oxidises the polysaccharide. This degradation can be enhanced by the presence of a reducing agent, such as ascorbic acid. In this study , the effect the iron(II) concentration on the hydroxyl radical-mediated degradation of beta-glucan was investigated and identified the intermediate species involved in the formation of hydroxyl radicals.

An increase in the iron(II) concentration did not have a significant effect on the degradation in the presence of a reducing agent (ascorbic acid), while in the mere presence of iron(II) it accelerates the degradation. The addition of catalase and superoxide dismutase (SOD) prevented the hydroxyl radical driven-degradation of beta-glucan induced by iron(II) or ascorbic acid/iron(II), demonstrating the involvement of both superoxide and hydrogen peroxide in the hydroxyl radical formation.

SOD, which catalyses the dismutation of superoxide into hydrogen peroxide, should have stimulated the formation of radicals, since these radicals are generated from the reaction between hydrogen peroxide and iron(II). In the present study, we hypothesise the mechanism of the inhibition of beta-glucan degradation by superoxide dismutase. (Faure *et al*, 2013)

j. Hydrolysis of β-glucan

The acid and enzymatic hydrolyses of oat β-glucan were compared for commercial oat β-glucan and β-glucan isolated from oat grain and oat bran. The resulting mono- and oligosaccharides were analysed by high-performance anion-exchange chromatography, combined with pulsed amperometric detection. The acid hydrolysis was studied with HCl, TFA and H2SO4 at two concentrations, with three durations of hydrolysis and at three temperatures.

Under the mildest acid conditions (37ºC and pH 1, corresponding to those in the human stomach) no degradation of β-glucan was observed with HCl over a 12 h period. At 120 °C, total hydrolysis to glucose occurred with high-concentration acids. Total recovery was also achieved with lichenase hydrolysis.

Hydrolysis, with lichenase and β-glucosidase together, produced glucose, which was analysed spectrophotometrically according to the AOAC 995.16 method. Since the results with the original method were not satisfactory, the method was further modified to determine the best conditions for samples with high β-glucan content and poor solubility. (Johanssona *et al*,2006)

III. METHODOLOGY

Methodology is the systematic, theoretical analysis of the methods applied to a field of study. The methodology of the study entitled ***'Estimation and Profiling of Beta Glucan in Selected Millets'*** is described under the following headings:

A. Selection and collection of samples
B. Preparation of samples
    1) Raw sample
    2) Soaked sample
    3) Sprouted sample
    4) Pressure cooked sample
C. Estimation of samples for β-Glucan
D. Purification of β-Glucanase
E. Estimation of β-Glucan in selected millets

Glucans are glucose polymers, classified according to their interchain linkage as being either α- or β-linked . β-glucans are a heterogeneous group of nonstarch polysaccharides, consisting of D-glucose monomers linked by β-glycosidic bonds. The macromolecular structure of β-glucan depends on both the source and method of isolation. (Barsanti *et al,* 2011)

β-glucan is a relatively inexpensive milling byproduct, and it is added to foods on the assumption that this will contribute to health benefits. β-glucans are predominantly found in the internal aleurone and subaleurone cell walls . The content of β-glucan varies with environmental conditions during endosperm development and is regulated by (1→3, 1→4)-β-glucan endohydrolase (EC 3.2.1.73 also known as licheninase or 1,3-1,4-beta glucanase) to facilitate endosperm cell-wall degradation during germination. (Khoury *et al,* 2011)

RESEARCH DESIGN

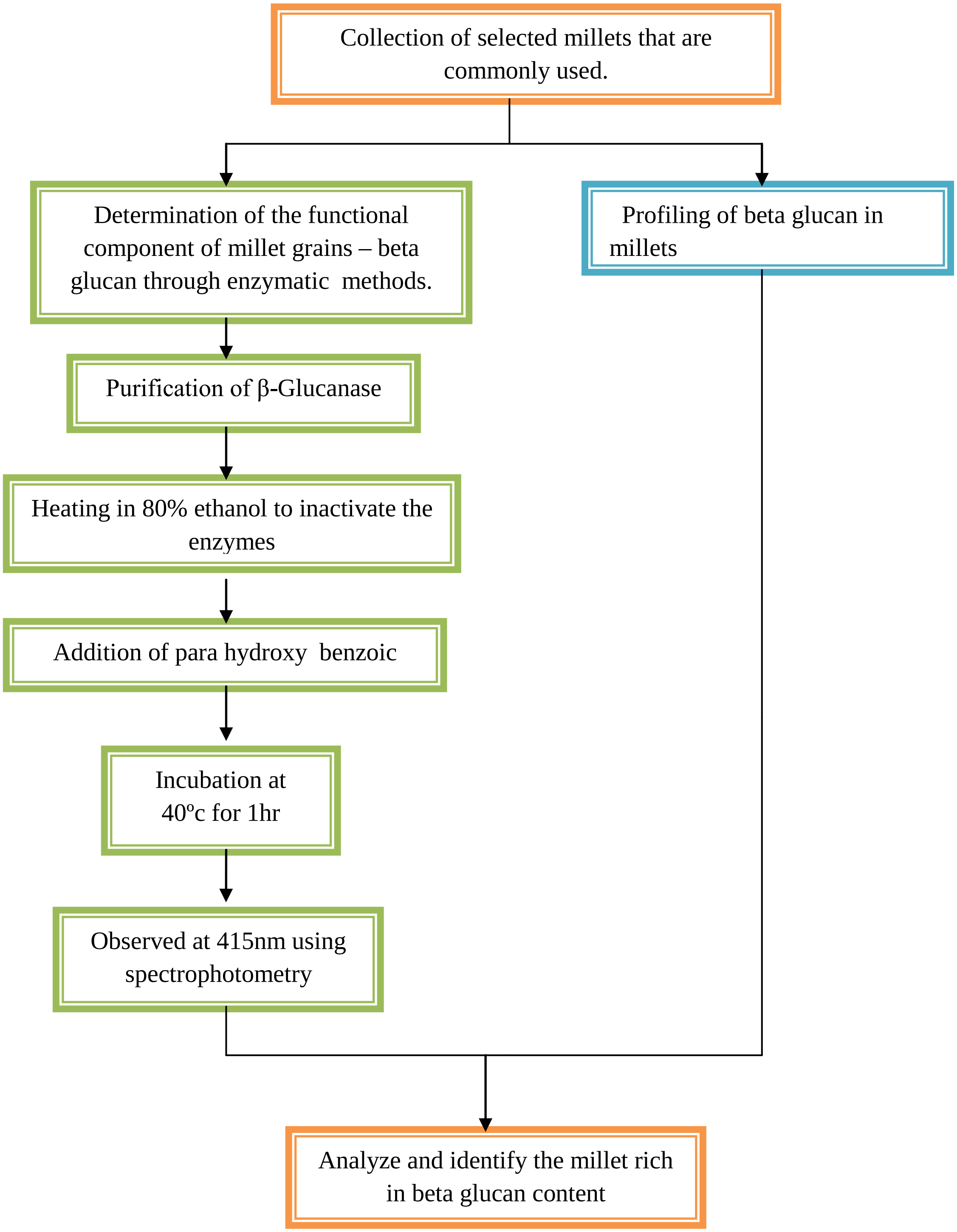


Figure 1

A. SELECTION AND COLLLECTION OF MILLETS:

Millets are tiny in size, round in shape and minor cereals of the small seeded-grass family (Poaceae). It is characterized by their remarkable ability to survive in less fertile soil, drought-resistant, resistance to pests and diseases, short growing season. (Devi. *et al*, 2011) The millets chosen for the study are ;

Table I

Millets selected for the study

The below mentioned table enlists the Millets selected for the study and also its regional and scientific name.

S.No	Common Name	Regional Name (Tamil)	Scientific Name
1.	Sorghum (Red, white)	Cholam	*Sorghum bicolor*
2.	Pearl millet (Naatu, Gundu)	Kambu	*Pennisetum glaucum*
3.	Finger millet	Kezhvaragu/Ragi	*Eleusine coracana*
4.	Proso millet	Panivaragu	*Panicum miliaceum*
5.	Kodo millet	Varagu	*Paspalum scrobiculatum*
6.	Foxtail millet	Thinai	*Setaria italic*
7.	Banyard millet	Kuthiravali	*Echinochloa esculenta*
8.	Little millet	Samai	*Panicum sumatrense*

The widely consumed millets like Cholam (Red, White), Kambu(Naatu, Gundu), Varagu, Varagu, Ragi, Thinai, Kuthiravali and Panivaragu were purchased from a Departmental store on 26.1.19. Thirty seven samples were prepared from the millets purchased. (Raw-10; Soaked-10; Sprouted-7 and Pressure cooked-10). The millets were tested for the β glucan content in raw, soaked, sprouted and pressure cooked form to assess the impact of various process on the level of β glucan.

Ethical clearance certificate is enclosed in the Appendix I.

B. PREPARATION OF SAMPLES:

1) Raw samples of selected millets:

For the raw sampling, 10g of each millet (Cholam (Red, White),Ragi, Kambu(Naatu, Gundu), Thinai, Panivaragu, Varagu, Kuthiravali and Samai) which did not undergo any cooking process was weighed in dry form and was subjected to further experimental process.

2) Soaked sample of selected millets:

For the soaked sample 10g of each sample (Cholam (Red, White),Ragi, Kambu(Naatu, Gundu), Thinai, Panivaragu, Varagu, Kuthiravali and Samai) was weighed and washed thoroughly to remove the impurities and soaked overnight (12 hours) in room temperature water in separate stainless steel bowls along with lids and then drained for the soaked samples. Millets like Cholam (Red, white), Naatu kambu and Ragi were soaked for (15 hours) since it is a little hard in nature when compared to other millets and their outer covering is thick and fibrous .

Soaking of grains is popular and household food processing technique. It is used for reducing antinutritional compounds like phytic acid and phytase activity to improve bioavailability of minerals (Lestienne *et al*, 2005).

3) Sprouted sample of selected millets:

Sprouting or germination is a process of planting through the germ layer. Sprouting increases the nutritive value as it enhances the fibre and vitamin-C content.

Germination of millets (Pennisetum typhoides) also decreased the levels of tannins (1.6% to 0.83%). Germination improved the in vitro protein (14% to 26%) and starch (86% to 112%) digestibility in pearl millet (Shahidi *et al*, 2013). It also led to the reduction of anti-nutrients such as phytic acid, tannins, and polyphenols, which form complexes with protien. (Hassan *et al*, 2006)

For the germination, each millet was weighed, washed , drained and soaked overnight in water in separate bowls. Millets like Cholam (Red, White),Ragi, Kambu(Naatu, Gundu) were soaked for 15 hours in room temeperature and the other millets (Thinai, Panivaragu, Varagu, Kuthiravali and Samai) were soaked for 12 hours in room temperature water as they are termed as minor millets and are small in diameter and has a thin outer layer.

Then on the next morning the millets were drained and were tied in a muslin cloth to remove excess water and left undisturbed in a warm place for a day to germinate. Among the millets purchased Kodo, Banyard and Little millet did not sprout as the germ was removed before it is sold. And the millet Panivaragu gained a little extra time to germinate as it had a small germ layer.

4) Pressure cooked sample of selected millets:

Pressure cooking is the process of cooking under pressure by trapping the steam produced, so that the water reaches 100 ºc and reduces the cooking time. All the millets were weighed, washed and pressure cooked in a cooker along with 500ml water separately and cooled.

Sorghum (Red, White), Pearl millet (Naatu, Gundu) and Ragi were pressure cooked for 20 minutes and 5 whistles. These major millets comparatively took more time to cook as they are fibruos and have harder exosperm and pericarp layer.

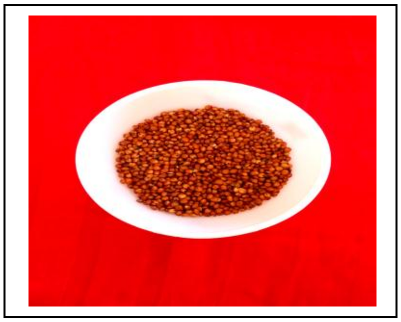
Banyard millet, Foxtail millet, Little millet, Kodo millet and Proso millet were pressure cooked for 10 minutes and 3 whistles. These are termed minor millets and are seed like in structure and hence got cooked and became soft quickly.

Table II

Time taken for each millet to cook under pressure

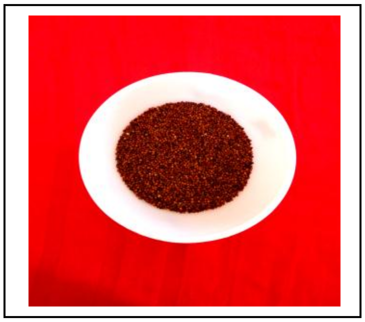
The below mentioned table reports the time consumed for each type of millet to cook under pressure

Type of millet	Cooking time (minutes)	No. of whistles
Sorghum (red, white) *(Sorghum bicolor)*	20	5
Pearl millet (naatu, gundu) *(Pennisetum glaucum)*	20	5
Finger millet *(Eleusine coracana*)	20	5
Banyard millet *(Echinochloa esculenta)*	10	3
Little millet (*Panicum sumatrense)*	10	3
Kodo millet *(Paspalum scrobiculatum)*	10	3
Proso millet (*Pannicum miliacium)*	10	3
Foxtail millet (*Setaria italic*a)	10	3



Sorghum bicolor (Red)
(Cholam)

Sorghum bicolor (White)
(Cholam)



Eleusine coracana
(Kezhvaragu)

Pennisetum glaucum
(Gundu kambu)

Pennisetum glaucum
(Naatu kambu)

Setaria italica
(Thinai)

Panicum miliaceum
(Panivaragu)

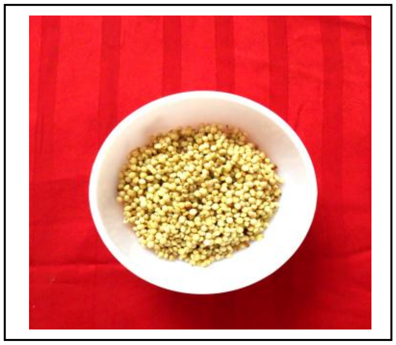
Paspalum scrobiculatum
(Varagu)

Echinochloa esculenta
(Kuthiravali)

Panicum sumatrense
(Samai)

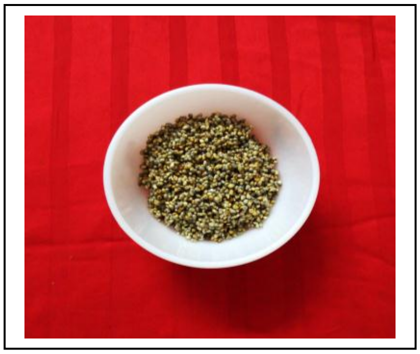
Plate I (Raw samples of selected Millets)

Sorghum bicolor (Red)
(Cholam)

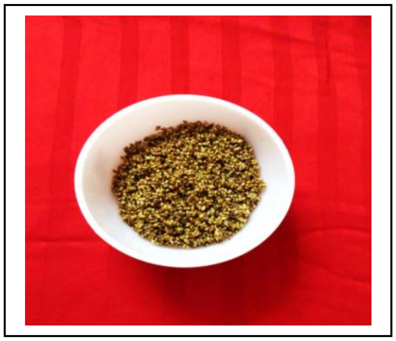


Sorghum bicolor (White)
(Cholam)

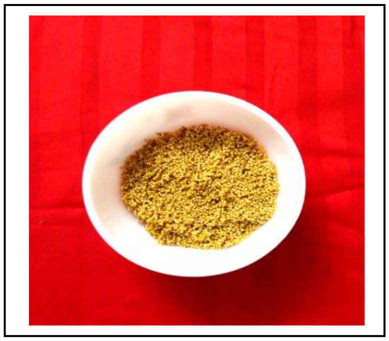
Eleusine coracana
(Kezhvaragu)



Pennisetum glaucum
(Gundu kambu)



Pennisetum glaucum
(Naatu kambu)



Setaria italica
(Thinai)

Panicum miliaceum
(Panivaragu)

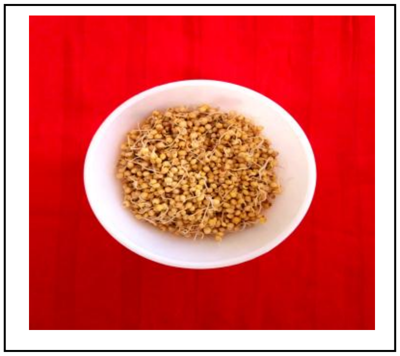
Paspalum scrobiculatum
(Varagu)

Echinochloa esculenta
(Kuthiravali)

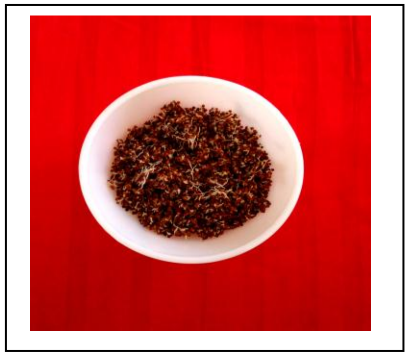
Panicum sumatrense
(Samai)

Plate II (Soaked samples of selected Millets)

Sorghum bicolor (Red)
(Cholam)



Sorghum bicolor (White)
(Cholam)

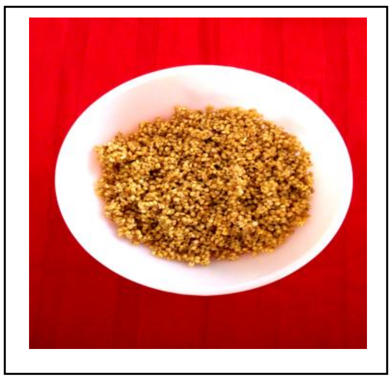


Eleusine coracana
(Ragi)

Pennisetum glaucum
(Gundu kambu)

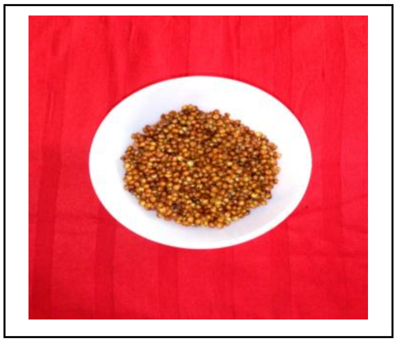
Pennisetum glaucum
(Naatu kambu)

Setaria italica
(Thinai)

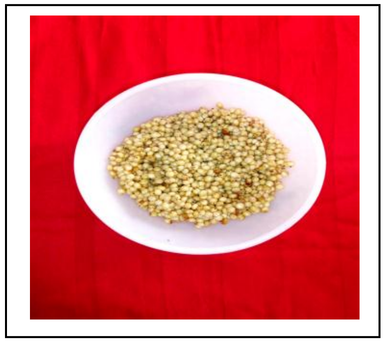


Panicum miliaceum
(Panivaragu)

Plate III (Sprouted samples of selected Millets)

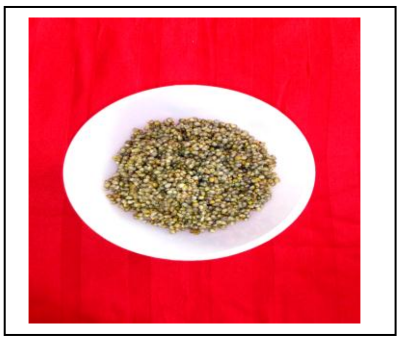


Sorghum bicolor (Red)
(Cholam)

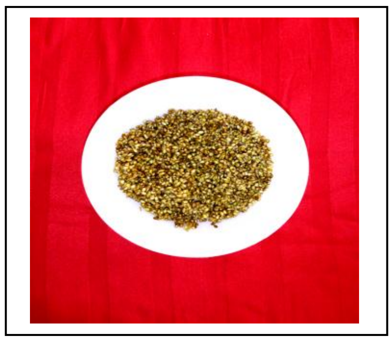


Sorghum bicolor (White)
(Cholam)

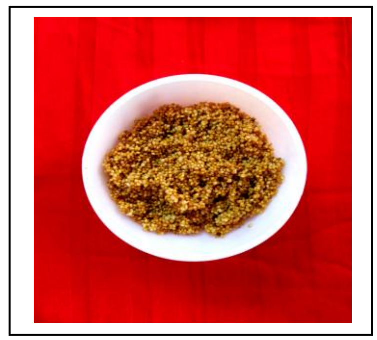
Eleusine coracana
(Kezhvaragu)



Pennisetum glaucum
(Gundu kambu)



Pennisetum glaucum
(Naatu kambu)



Setaria italica
(Thinai)

Panicum miliaceum
(Panivaragu)

Paspalum scrobiculatum
(Varagu)

Echinochloa esculenta
(Kuthiravali)

Panicum sumatrense
(Samai)

Plate IV (Pressure cooked samples of selected Millets)

Then each sample (37) (Raw-10; Soaked -10; Sprouted -7; Pressure cooked -10) was air tightly packed in zip lock covers and then labeled based on the processing undergone .

C. ESTIMATION OF SAMPLES FOR β-GLUCAN:

Enzymatic method is used for the estimation of β-glucan and was calibrated spectrophotometrically .The following are the procedures involved,

AIM:

To estimate the amount of β-glucan present in the widely used millets by enzymic method

PRINCIPLE:

Inactivating enzymes by heating in 80% ethanol . β -glucan is hydrolysed by incubating for 1hour with high concentration of purified β –glucanase to determine the reducing sugars, by which β –glucan content is estimated.

APPARATUS & EQUIPMENTS REQUIRED:

- Centrifuge
- Incubator
- Spectrophotometer
- Weighing machine
- Test tubes
- Centrifuge tubes
- Sieve
- Standard flask
- Beakers
- Glass rod
- Bunsen burner
- Water bath bowl

REAGENTS REQUIRED:

- Bacterial amylase
- 50 mM Phosphate buffer
- DEAE-Cellulose

- 100mMacetate buffer
- 0.5% $CaCl_2$
- Ethanol
- Starch
- 50mM Sodium acetate buffer
- *p*-hydroxybenzoic acid hydrazide (PAHBAH)
- Distille

D. PURIFICATION OF β-GLUCANASE:

The β-glucanase contaminant in a commercial amylase was purified. Bacterial amylase was stirred in 500ml of 50 mM phosphate buffer, pH 8.0. The suspension was centrifuged at 4000g for 5minutes. The supernatant was added to a bed of DEAE- cellulose (50g of Whatman DE52 that had been equilibrated with 50mM phosphate buffer, pH 8.0.

The β-glucanase was eluted with 100m Macetate buffer pH 4.0. the first 165ml was discarded and the next 400ml was collected. This fraction contained 22% of the original β-glucanase but only 0.6% of the α-amylase. The remaining α-amylase was removed by precipitation with starch. This was achieved by adding 0.5% $CaCl_2$,40% (v/v) ethanol and 20% (w/v) starch .The mixture was stirred and centrifuged at 4,000g for 10 minutes.

This procedure did not decrease the β-glucanase activity but removed 99.7% of the remaining α-amylase. The last traces of α-amylase were removed by a second precipitation with starch. The final precipitation produced 3.4μ moles of reducing sugar/sec/ml when assayed at 40 ºc and pH 5.0 with 1mg/ml as substrate.

E. PROCEDURE FOR β-GLUCAN ESTIMATION:

Each millet was ground in a laboratory mill to pass a 0.8mm sieve. The ground sample (0.250g) was weighed directly into 10ml centrifuge tubes. Endogenous enzymes were inactivated by adding 5ml of 80% v/v ethanol and boiling gently on a water bath for 5 minutes. The mixture was centrifuged at 3,000g for 5 minutes and the supernatant was discarded. The pellet was washed with 5ml of 80%v/v ethanol.

The washed sample was then suspended in 5ml of 50mM Sodium acetate buffer pH5.0 and 0.5ml of the purified β-glucanase solution and incubated at 40ºc for 1 hour. Following centrifugation , the reducing sugars released were measured by reaction with *p*-hydroxybenzoic acid hydrazide (PAHBAH) a control in which the β-glucanase was replaced with distilled water was included for each millet sample.

Hence the beta-glucan content of each millet under different processing were estimated and analysed.

Source: Henry, (1984), A simplified enzymic method for the determination of (1-3)(1-4)-β-glucans in barley. J. Inst.Brew.,May,(90),178-180

β-glucan Assay

↓

Ground and sieved each millet in 0.8mm sieve

↓

0.0250g ground sample was weighed into centrifuge tube

↓

5ml of 80% ethanol was added to inactivate the enzymes and kept in water bath for 5 minutes

↓

Centrifuged at 3000g x 5minutes and discarded the supernatant

↓

Formed pellet was washed with 5ml of 80% ethanol

↓

Suspended in 5ml of 50mMSodium acetate buffer , 0.5ml purified β-glucanase solution and adjusted to pH5.0

↓

Incubated 40^{o}c for 1 hour

↓

Bacterial amylase was stirred in 500ml of 50 mM phosphate buffer at pH 8.0

↓

Centrifuged at 4000g x 5minutes and added to a bed of DEAE- cellulose

↓

β-glucanase was eluted with 100mMacetate buffer at pH 4.0

↓

Discarded the supernatant and 400ml was collected

↓

Remaining amylase was removed by precipitation of starch By adding 0.5%$CaCl_2$, 40% (v/v) ethanol and 20% (w/v) starch

↓

Mixture was centrifuged at 4000g x 10minutes

↓

Series of precipitation was done to produce 3.4µ moles of reducing sugar/sec/ml

↓

Assayed at 40 ^{o}c and pH 5.0 with 1mg/ml as substrate

↓

Reducing sugars where measured by the reaction with PAHBAH (β-glucanase was replaced with distilled water)

↓

Reading was taken in spectrophotometer

Figure 2 : Schematic representation of experimental

Statistical Analysis was done using the SPSS software Version 21. The beta glucan levels were repeated for each of the processed millet as duplicates and the data are presented as mean + Standard deviation (SD). Data were subjected to one way analysis of variance ANOVA representing the 1) between different millets in a particular process 2) between different processes for a particular millet type. Significant data were further analysed by Post-Hoc test of Fisher's Least Square Deviation at 5% and 1% to differentiate the mean.

Two way analysis of variance (ANOVA) and the means were separated using Least Significant Difference (LSD) This Two way ANOVA analysis was carried out using the general liner regression modeling with the dependent variable as beta glucan content and the independent fixed factors as millet types and different processing methods the significance at various levels of millet types, processing methods and their interaction.

Hypothesis of the study:

H_{1}: Millets do not contain β-glucan.

H_{2}: There is no effect of the processing method on the beta glucan content

IV. RESULTS AND DISCUSSION

The results pertaining to the study entitled ***'Estimation and Profiling of Beta Glucan in Selected Millets'*** is discussed in this part of the study. This study was conducted to estimate the Beta glucan (a vital soluble dietary fibre) content in commonly consumed millet grains under various processing such as Soaked, Sprouted and Pressure cooked in comparison with the Raw samples.

Enzymatic method was followed to estimate the beta-glucan content and was read in spectrophotometer. Also the main aspect of the study was to ensure if the processing or the pre-processing of the millet grains had any impact over the quantity of beta-glucan.

The results obtained are discussed under the following headings and they are as follows,

A. Estimated amount of Beta-glucan in selected millets
B. Estimation of Beta-glucan within the selected millets
C. Estimation of Beta-glucan within the selected Process
D. Effect of Processing on β-glucan content of selected millets
E. Combined Estimation
F. β-glucan content in selected millets.

A. ESTIMATED AMOUNT OF BETA-GLUCAN IN SELECTED MILLETS:

Table III

Amount of beta-glucan present in Raw sample of selected millets (in %)

This table elaborates on the percentage of beta-glucan present in Raw form of selected millets which did not undergo any processing.

S.No	Type of Millet	β- Glucan %
1.	Thinai (*Setaria italica*)	4.7
2.	Varagu (*Paspalum scrobiculatum*)	4.5
3.	Ragi (*Eleusine coracana*)	3.0
4.	Pani varagu (*Panicum miliaceum*)	1.8
5.	Samai (*Panicum sumatrense*)	1.8
6.	Kuthiravali (*Echinochloa esculenta*)	1.6
7.	White cholam (*Sorghum bicolor*)	1.3
8.	Red cholam (*Sorghum bicolor*)	1.2
9.	Gundu kambu (*Pennisetum glaucum*)	1.0
10.	Naatu kambu (*Pennisetum glaucum*)	0.8
11.	Oats (*Avena sativa)*	3.29 (Sterna *et al*,2015)

Here the millets were examined in the raw form, Through Table III we can understand that the millet *Setaria italica (*Thinai) has the highest beta-glucan content by nature and *Pennisetum glaucum* (Naatu kambu) had the least amount of beta –glucan. The order of heirarchy is observed as Thinai, varagu, Ragi, Pani varagu, Samai, Kuthiravali, White cholam, Red cholam, Gundu kambu and Naatu kambu.

SOAKED SAMPLE OF SELECTED MILLETS

Table IV

Amount of beta-glucan present in Raw sample of selected millets (in %)

The following table reveals the amount of beta-glucan present in soaked samples of selected Millets in percentage.

S.No	Type of Millet	β- Glucan %
1.	Varagu (*Paspalum scrobiculatum*)	5.0
2.	Thinai (*Setaria italica*)	4.8
3.	Ragi (*Eleusine coracana*)	3.5
4.	Pani varagu (*Panicum miliaceum*)	2.2
5.	Samai (*Panicum sumatrense*)	2.2
6.	Kuthiravali (*Echinochloa esculenta*)	2.1
7.	White cholam (*Sorghum bicolor*)	1.7
8.	Red cholam (*Sorghum bicolor*)	1.6
9.	Gundu kambu (*Pennisetum glaucum*)	1.4
10.	Naatu kambu (*Pennisetum glaucum*)	1.2

By the evidence of Table IV we can infer that soaking has minor contribution in increasing the beta-glucan content of millets . Here the highest amounted of beta-glucan is recorded by *Paspalum scrobiculatum* (varagu) and the least amount by *Paspalum scrobiculatum* (Naatu kambu).

In this process the beta glucan content gradually reduces as follows; Varagu, Thinai Ragi, Pani varagu, Samai, Kuthiravali, White cholam, Red cholam, Gundu kambu and Naatu kambu.

Hence the soaking process does not enhance the beta glucan levels to a commendable rate, but can be applied when compared to the beta-glucan content of the raw millet grains.

SPROUTED SAMPLE OF SELECTED MILLETS:

Table V

Amount of Beta-glucan present in sprouted samples of selected millets (in %):

The below mentioned table shows the amount of beta-glucan present in sprouted samples of selected Millets in percentage.

S.No	Type of millet	β- Glucan %
1.	Thinai (*Setaria italica*)	6.5
2.	Ragi (*Eleusine coracana*)	4.5
3.	Pani varagu (*Panicum miliaceum*)	2.7
4.	Red cholam (*Sorghum bicolor*)	2.4
5.	White cholam (*Sorghum bicolor*)	2.3
6.	Gundu kambu (*Pennisetum glaucum*)	2.1
7.	Naatu kambu (*Pennisetum glaucum*)	1.7

According to above table (Table V) the process of germinating has remarkbly increased the beta glucan content of the subjected millet grains. Here *Setaria italica (*Thinai) has the highest amount of beta glucan and the lowest content is recorded in the *Paspalum scrobiculatum* (Naatu kambu).

In the process of germination the beta glucan content is observed to gradualy decrease as follows; Thinai, Ragi, Pani varagu, Red cholam, White cholam, Gundu kambu and Naatu kambu.

Compared to the above mentioned samples, The process of germination is found to increase the beta-glucan levels the most.

PRESSURE COOKED SAMPLE OF SELECTED MILLETS:

Table VI

Amount of Beta-glucan present in sprouted samples of selected millets (in %):

This table shows the amount of beta-glucan present in Pressure cooked samples of selected millets in percentage.

S.No	Type of Millet	β- Glucan %
1.	Thinai (*Setaria italica)*	7.0
2.	Varagu (*Paspalum scrobiculatum*)	6.5
3.	Ragi (*Eleusine coracana*)	5.2
4.	Kuthiravali (*Echinochloa esculenta*)	4.4
5.	Pani varagu (*Panicum miliaceum*)	3.3
6.	Samai (*Panicum sumatrense*)	3.1
7.	Red cholam (*Sorghum bicolor*)	2.8
8.	Gundu kambu (*Pennisetum glaucum*)	2.7
9.	White Cholam (*Sorghum bicolor*)	2.6
10.	Naatu kambu (*Pennisetum glaucum*)	2.5

On observing the data of Table VI we can understand that the process of Pressure cooking has the maximum effect on predominanty increasing the beta-glucan conten in millet grains among the processing methods used .

Based on the above table (Table VI) *Setaria italica (*Thinai) is the millet with the highest amount of beta-glucan. And the order of presence gores by Thinai, varagu, Ragi, Pani varagu, Samai, Kuthiravali, White cholam, Red cholam, Gundu kambu and Naatu kambu.

Based on the results Pressure cooking is the best method of processing / cooking the milets to increase its beta-glucan content and there by increase the fibre content and also the other health promoting properties.

B. Estimation of beta-glucan within the selected Millets:

Table VII

Significance of beta glucan level among the different types of millets

The following table discusses about the ranking among the selected millets on comparing with one type of millet.

Millet	**Estimate**
Thinai (*Setaria italica*)	5.682 ± 0.81
Varagu (*Paspalum scrobiculatum*)	5.307 ± 0.93
Ragi (*Eleusine coracna*)	4.098 ± 0.81
Panivaragu (*Panicum miliaceum*)	2.700 ± 0.81
Samai (*Panicum sumatrense*)	2.467 ± 0.93
Kuthiravali (*Echinochloa esculenta*)	2.518 ± 0.93
White cholam (*Sorghum bicolor*)	2.025 ± 0.81
Red cholam (*Sorghum bicolor*)	2.025 ± 0.81
Gundu kambu (*Pennisetum glaucum*)	1.791 ± 0.81
Naatu kambu (*Pennisetum glaucum*)	1.588 ± 081

From the above table we can understand that there is no much significant difference in level of beta-glucan among the different kinds of millets. And the ranking of millets goes by Thinai, Varagu, Ragi Panivaragu, Samai, Kuthiravali, White cholam, Red cholam ,Gundu kambu and Naatu kambu.

C. Estimation of beta-glucan within the selected Process:

Table VIII

Significance of beta glucan level among the different types of processing method

The following table discusses about the ranking among the method of processing applied on the selected millets.

Processing	Estimation
Pressure cooked	4.011 ± 0.051
Sprouted	3.179 ± 0.061
Soaked	2.607 ± 0.051
Raw	2.186 ± 0.051

From the above table, we can understand that the process of Pressure cooking increases the level of beta-glucan followed by sprouting, soaking, and raw samples.

D. Effect of processing on beta-glucan content of selected millets:

Table IX

Effect of Processing on the availability of beta glucan content in different millets

The following table speaks about the impact of processing on the beta-glucan content of each millet.

Grains / Processing Method ▪	Thinai	Varagu	Ragi	Pani varagu	Samai	Kuthiravali	White Cholam	Red Cholam	Gundu Kambu	Naatu Kambu
Raw	**4.69 ± 0.09**	**4.43 ± 0.11**	**3.0 ± 0.07**	**1.85 ± 0.06**	**1.89 ± 0.13**	**1.53 ± 0.10**	**1.27 ± 0.05**	**1.31 ± 0.16**	**1.01 ± .01**	**0.90 ± 0.14**
Soaked	**4.83 ± 0.04**	**5.08 ± 0.11[a]**	**3.46 ± 0.06 [a]**	**2.26 ± 0.08**	**2.35 ± 0.21**	**2.25 ± 0.21**	**1.73 ± 0.04[a]**	**1.58 ± 0.03[a]**	**1.38 ± 0.04 [a]**	**1.18 ± 0.04[a]**
Pressure cooked	**6.79 ± 0.30[ab]**	**6.42 ± 0.11[ab]**	**5.35 ± 0.21 [ab]**	**3.95 ± 0.64 [ab]**	**3.16 ±0.20[ab]**	**3.78 ± 0.95**	**2.71 ± 0.13 [ab]**	**2.76 ± 0.08 [ab]**	**2.67 ± 0.10 [ab]**	**2.53 ± 0.04[ab]**
Sprouted (SP)	**6.26 ± 0.37[abc]**	**-**	**4.53 ± 0.04 [abc]**	**2.75 ± 0.07 [abc]**	**-**		**2.40 ± 0.14 [abc]**	**2.45 ± 0.07 [abc]**	**2.11 ± 0.01 [abc]**	**1.75 ±0.07[abc]**
P value										

One Way ANOVA, Values are expressed as **Mean ± S.D**, p values significant at **p< 0.05** for one way ANOVA (▪), ‘**a**’ denotes significant difference at 5% or 1% level between Raw and Soaked, Pressure cooked and Sprouted;‘**b**’ denotes significant difference at 5% or 1% level between Soaked with Pressure cooked and Sprouted ;‘ **c**’ denotes significant difference at 5% or 1 % level between Pressure cooked and Sprouted. Superscripts (a,b,c) indicates the significant difference between the processing methods within a millet.

From the table IX it is clear that beta glucan content was found to vary significantly ($p < 0.05$) in each processing methods of cereal grains except for kuthiravali grains (*Echinochloa esculenta*). Pressure cooked foxtail grain was found to contain the highest beta glucan content of about 6.79 % followed by germinated grains with 6.26 % of in comparison to the raw grains. Reports are available that states the higher levels of beta glucan in various hydrothermal processing methods of food preparation. Similar trend was also observed in Pani varagu grain (*Panicum miliaceum*). No significant difference was obtained between the soaked thinai (*Setaria itlica*), pani varagu(*Panicum miliaceum*) and samai (*Panicum* sumatrense)in comparison to the raw grain.

This highlights that there is no influence on beta glucan content by soaking method among these grains. In contrast to this, soaking method was found to positively influence the beta glucan levels in varagu (*Paspalum scrobiculatum*), ragi (*Eleusine coracana*), white cholam (*Sorghum bicolor*), red cholam (*Sorghum bicolor*), gundu kambu (*Pennisetum glaucum*) and naatu kambu (*Pennisetum glaucum*). Among all the processing methods, selected pressure cooked grains were found to have highest levels of beta glucan except in kuthiravali grains (*Echinochloa esculenta*). The sprouted grains of kuthiravali were found to have highest beta-glucan in contrast to the other grains in which the pressure cooked grains had higher content. Also the raw grains were found to have the least content of beta glucan. Hence processing influences the beta-glucan content to various levels.

i) Beta glucan levels available in different millets by a particular processing method (in %):

Table X

Significance of processing method on the beta-glucan availability of millets

This table illustrates the significance of processing on the beta-glucan content of selected millets.

Processing / Millet	Raw	Soaked	Pressure cooked	Sprouted
Thinai	4.69 ± 0.09	4.83 ± 0.04	6.79 ± 0.30	6.26 ± 0.37
Varagu	4.43 ± 0.11	5.08 ± 0.11	6.42 ± 0.11	-
Ragi	3.0 ± 0.07	3.46 ± 0.06	5.35 ± 0.21	4.53 ± 0.04
Pani varagu	1.85 ± 0.06	2.26 ± 0.08	3.95 ± 0.64	2.75 ± 0.07
Samai	1.89 ± 0.13	2.35 ± 0.21	3.16 ±0.20	-
Kuthiraivali	1.53 ± 0.10	2.25 ± 0.21	3.78 ± 0.95	-
White Cholam	1.27 ± 0.05	1.73 ± 0.04	2.71 ± 0.13	2.40 ± 0.14
Red Cholam	1.31 ± 0.16	1.58 ± 0.03	2.76 ± 0.08	2.45 ± 0.07
Gundu kambu	1.01 ± .01	1.38 ± 0.04	2.67 ± 0.10	2.11 ± 0.01
Naatu kambu	0.90 ± 0.14	1.18 ± 0.04	2.53 ± 0.04	1.75 ±0.07
p Value	0.05	0.05	0.05	0.05

The magnitude of beta glucan in different cereal grains were as follows: thinai was found to have the significantly highest beta glucan content followed by ragi (*Eleusine coracana*), samai (*Panicum sumatrense*), pani varagu (*Pennisetum miliaceum*), kuthiravali (*Echinochloa esculenta*), red cholam (*Sorghum bicolor*), white cholam (*Sorghum bicolor*), gundu kambu (*Pennisetum glaucum*) and naatu kambu (*Pennisetum glaucum*) among all the grains (4.64 %, 3.05 %, 1.89%, 1.85 %, 1.53 %, 1.31 %, 1.27%, 1.01 % and 0.90 %. Raw Varagu was found to contain beta glucan level (4.43%) that did not vary significantly with the raw thinai grain in contrast to other grains. Among the soaked grains, varagu was found to have significantly higher levels of beta glucan (5.08%) followed by thinai (4.83 %), ragi (3.46%), samai (2.35%) pani varagu (2.26%), kuthiravali (2.25), white cholam (1.73%), red cholam (1.58%), gundu kambu (1.38%) and naatu kambu (1.18%). Among the pressure cooked grains, the beta glucan was found to be significantly highest in thinai (6.79%)

followed by varagu (5.3%), ragi (3.95%), panivaragu (3.95%), kuthiravali (3.78%),samai (3.16), red cholam (2.76%), white cholam (2.71%), gundu kambu (2.67%) and naatu kambu (2.53%). The beta glucan content was found to be significantly highest in thinai (6.26%) followed by ragi (4.53%), kuthiravali (4.45%), pani varagu (2.75%), red cholam (2.4%), white cholam (2.40%),gundu (2.11%), and naatu (1.75%).

E. COMBINED ESTIMATION:

Table XI

Consolidation Based on both effect of processing and type of millet

The below mentioned table contains the consolidated data of both effect of processing and kind of millet.

Grains / Processing Method	Thinai	Varagu	Ragi	Pani varagu	Samai	Kuthiravali	White Cholam	Red Cholam	Gundu Kambu	Naatu Kambu
Raw	**4.69 ± 0.09**	**4.43 ± 0.11**	**3.0 ± 0.07**	**1.85 ± 0.06**	**1.89 ± 0.13**	**1.53 ± 0.10**	**1.27 ± 0.05**	**1.31 ± 0.16**	**1.01 ± .01**	**0.90 ± 0.14**
Soaked	**4.83 ± 0.04**	**5.08 ± 0.11^{a}**	**3.46 ± 0.06^{a}**	**2.26 ± 0.08**	**2.35 ± 0.21**	**2.25 ± 0.21**	**1.73 ± 0.04^{a}**	**1.58 ± 0.03^{a}**	**1.38 ± 0.04^{a}**	**1.18 ± 0.04^{a}**
Pressure cooked	**6.79 ± 0.30^{ab}**	**6.42 ± 0.11^{ab}**	**5.35 ± 0.21^{ab}**	**3.95 ± 0.64^{ab}**	**3.16 ±0.20^{ab}**	**3.78 ± 0.95**	**2.71 ± 0.13^{ab}**	**2.76 ± 0.08^{ab}**	**2.67 ± 0.10^{ab}**	**2.53 ± 0.04^{ab}**
Sprouted	**6.26 ± 0.37^{abc}**	-	**4.53 ± 0.04^{abc}**	**2.75 ± 0.07^{abc}**	-	-	**2.40 ± 0.14^{abc}**	**2.45 ± 0.07^{abc}**	**2.11 ± 0.01^{abc}**	**1.75 ±0.07^{abc}**
p value	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	**< 0.05**	**> 0.05**	**< 0.01**	**< 0.01**	**< 0.01**	**< 0.01**

Values are expressed as **Mean ± S.D**, p values significant at **p< 0.05** for one way ANOVA (▪), '**a**' denotes significant difference at 5% or 1% level between Raw and Soaked, Pressure cooked and Sprouted;'**b**' denotes significant difference at 5% or 1% level between Soaked with Pressure cooked and Sprouted ;' **c**' denotes significant difference at 5% or 1 % level between Pressure cooked and Sprouted.

The beta glucan content were observed in various cereal grains processed by different methods of food preparation. This is displayed in Table 5 From which it was clear that beta glucan content was found to vary significantly (p < 0.05) in each processing methods of cereal grains except for kuthiravali grains (*Echinochloa esculenta*). Pressure cooked foxtail grain was found to contain the highest beta glucan content of about 6.79 % followed by germinated grains with 6.26 % of in comparison to the raw grains. Reports are available that states the higher levels of beta glucan in various hydrothermal processing methods of food preparation.

Similar trend was also observed in Panivaragu grain (*Panicum miliaceum*). No significant difference was obtained between the soaked thinai, pani varagu and samai in comparison to the raw grain. This highlights that there is no influence on beta glucan content by soaking method among these grains. In contrast to this, soaking method was found to positively influence the beta glucan levels in varagu (*Paspalum scrobilatum*), ragi (*Eleusine coracana*), white cholam (*Sorghum bicolor*), red cholam (*Sorghum bicolor*), gundu kambu (*Pennisetum glaucum*) and naatu kambu (*Pennisetum glaucum*). Among all the processing methods, selected pressure cooked grains were found to have highest levels of beta glucan except in kuthiravali grains (*Echinichloa esculenta*). The sprouted grains of Thinai (*Setaria* italica) were found to have highest betaglucan content in contrast to the other grains in which the pressure cooked grains had higher content. Also the raw grains were found to have the least content of beta glucan. Hence processing influences the betaglucan content to various levels among all the selected grains. Pressure cooked grains were found to have highest level of beta glucan in all grains .

The magnitude of beta glucan in different cereal grains were as follows:

Thinai was found to have the significantly highest beta glucan content followed by ragi (*Eleusine coracana*), samai (*Panicum sumatrense*), panivaragu (*Panicum miliaceum*), kuthiraivali, red cholam, white cholam, gundu and naatu Kambu among all the grains (4.64 %, 3.05 %, 1.89%, 1.85 %, 1.53 %, 1.31 %, 1.27%, 1.01 % and 0.90 %. Raw Varagu (*Paspalum scrobilatum*) was found to contain beta glucan level (4.43%) that did not vary significantly with the raw thinai grain (*Setaria italica*) in contrast to other grains. Among the soaked grains, varagu was found to have significantly higher levels of beta glucan (5.08%) followed by thinai (4.83 %), ragi (3.46%), samai (2.35%) pani varagu (2.26%), kuthiraivaali (2.25), white cholam (1.73%), red cholam (1.58%), gundu (1.38%) and naatu (1.18%).

Among the pressure cooked grains, the beta glucan was found to be significantly highest in thinai (6.79%)followed by varagu (5.3%), ragi (3.95%), pani varagu (3.95%), kuthiraivali (3.78%),samai (3.16),red cholam (2.76%), white cholam (2.71%), gundu kambu (2.67%) and naatu kambu (2.53%). The beta glucan content was found to be significantly highest in thinai (6.26%) followed by ragi (4.53%),kuthiraivali (4.45%), pani varagu (2.75%), red cholam (2.4%), white cholam (2.40%),gundu kambu (2.11%), and naatu kambu (1.75%).

To conclude, the best processing method to increase the beta glucan, a vital soluble dietary fibre content is by pressure cooking followed by sprouting and soaking instead of using raw grains for food preparation. The highest beta glucan content was found in thinai (*Setaria italica*)in all methods of pressure cooking, sprouting and in raw grains whereas it was found to be less in soaked grains in comparison to the soaked varagu grains (*Paspalum scrobilatum*).

F. BETA-GLUCAN CONTENT IN SELECTED MILLETS

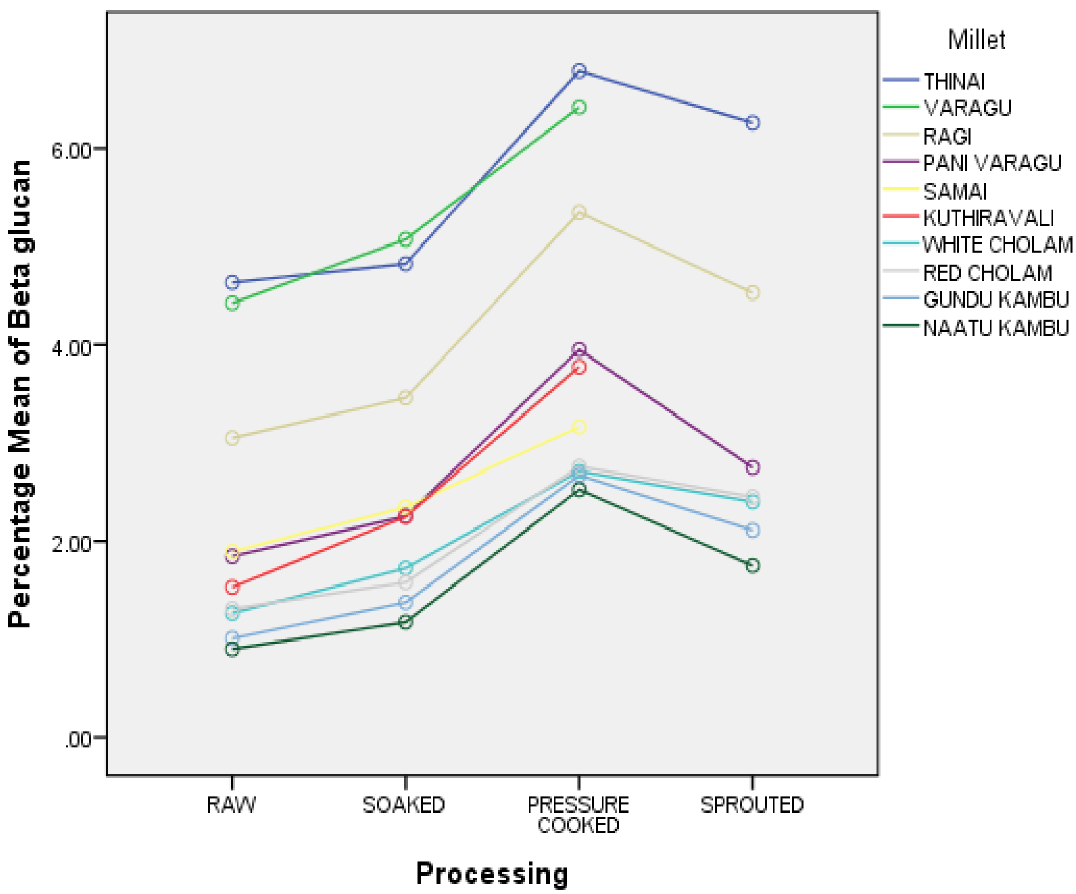


Figure 3: Estimated mean of beta-glucan (Process $v_{s.}$ Millet)

Therefore the hypothesis framed for the study entitled "*Estimation and Profiling of Beta-Glucan in Selected Millets"* were proven wrong as all the millets selected for the study contained a remarkable amount of beta glucan in its raw form. And the content of beta-glucan was enhanced further when it underwent a series of processing methods such as soaking, sprouting, and pressure cooking.

Among all the processing methods, pressure cooking method was recorded to increase the beta-glucan content to a large extent. Hence the study was concluded.

V. SUMMARY AND CONCLUSION

The study on ***"Estimation and Profiling of Beta-Glucan in Selected Millets"*** was taken up and conducted to find the actual beta-glucan content present in our regional food – Millets. And thereby to recommend millets and millet based foods for high fibre diet in order to treat chronic disorders such as diabetes mellitus, cardiovascular disorder and other non communicable diseases.

The main focus of the study was to estimate the amount of beta-glucan present in millets and the effect of processing over the content of beta-glucan.

Generally oats is regarded to be high in beta-glucan content but it is not our regional food and by keeping this fact in mind, the study was held to find out the cereal grain high in beta-glucan and focused in our traditional and regional food – Millets.

An elaborate study of the existing literature was done to find the commonly consumable millets, its production rate, nature of growth and their commendable health benefits.

Millets can be consumed by everyone as it is an easily available ingredient with minimum harvesting time and maximum production yield. Millets crops can also grow in infertile or swampy lands which makes it suitable for our tropical climatic conditions.

Beta-glucan is a soluble fibre found in most of the cereal grains and has also been approved as a functional component. Beta-glucan is supposed to have positive effect on human health as it has antioxidant, anti inflammatory properties and capacity to bind bile acid. Beta-glucan has been reported to treat a wide range of diseases such as hypercholesterolemia, obesity, diabetes mellitus ,cardiovascular disorders and also gastrointestinal disorders like constipation.

From the vast variety of millets 10 of them were selected for the study and they are Sorghum (Red, White) (*Sorghum bicolor*), Pearl millet (Naatu, Gundu) (*Pennisetum glaucum*), Finger millet (*Eleusine coracana*), Proso millet (*Panicum milliaceum*), Kodo millet (*Paspalum scrobiculatum*), Foxtail millet (*Setaria italica*), Banyard millet (*Echinochloa esculenta*), Little millet (*Panicum sumatrense*).

The selected millets were collected and 37 samples were prepared out of them such as Raw sample of Sorghum (Red, White) (*Sorghum bicolor*), Pearl millet (Naatu, Gundu) (*Pennisetum glaucum*), Finger millet (*Eleusine coracana*), Proso millet (*Panicum milliaceum*), Kodo millet (*Paspalumscrobiculatum*), Foxtail millet (*Setaria italica*), Banyard millet (*Echinochloa esculenta*), Little millet (*Panicum sumatrense*).

Soaked sample of Sorghum (Red, White) (*Sorghum bicolor*), Pearl millet (Naatu, Gundu) (*Pennisetum glaucum*), Finger millet (*Eleusine coracana*), Proso millet (*Panicum milliaceum*), Kodo millet (*Paspalumscrobiculatum*), Foxtail millet (*Setaria italica*), Banyard millet (*Echinochloa esculenta*), Little millet (*Panicum sumatrense*).

Sprouted sample of Sorghum (Red, White) (*Sorghum bicolor*), Pearl millet (Naatu, Gundu) (*Pennisetum glaucum*), Finger millet (*Eleusine coracana*), Proso millet (*Panicum milliaceum*), Foxtail millet (*Setaria italica*).

Pressure cooked sample of Sorghum (Red, White) (*Sorghum bicolor*), Pearl millet (Naatu, Gundu) (*Pennisetum glaucum*), Finger millet (*Eleusine coracana*), Proso millet (*Panicum milliaceum*), Kodo millet (*Paspalumscrobiculatum*), Foxtail millet (*Setaria italica*), Banyard millet (*Echinochloa esculenta*), Little millet (*Panicum sumatrense*).

After an extensive study, the enzymatic method of extraction was found to be the most effective method of extraction of beta-glucan when compared to other methods such as HPLC, enzymic, alkaline extraction. And hence the same method was followed in this study.

The samples were ground and sieved before being subjected to enzyme inactivation by 80% ethanol and then centrifuged at 3000g for 5 minutes. Further suspended in 5ml of 50mM Sodium acetate buffer,0.5ml purified β-glucanase solution at pH5.0 and incubated at 40ºc for 1 hour .

Followed by the addition of bacterial amylase in 500ml of 50mM phosphate buffer at pH 8.0 and again centrifuged at 4000g for 5minutes. Then β-glucanase was eluted with 100mM acetate at pH4.0 and finally centrifuged at 4000g for 10minutes.

The extract was analyzed at 40ºc and at pH5.0 with 1mg/ml of the substrate and the final reading was taken with the help of spectrophotometer at 415nm. And the percentage of beta-glucan present in each millet under different processing method are as follows, The Raw samples contained Foxtail millet (*Setaria italica*) – 4.7%, Kodo millet (*Paspalumscrobiculatum*)- 4.5%, Finger millet (*Eleusine coracana*)- 3.0%, Proso millet (*Panicum milliaceum*)- 1.8%, Little millet (*Panicum sumatrense*)- 1.8%, Banyard millet (*Echinochloa esculenta*)- 1.6%, Sorghum(White) (*Sorghum bicolor*)- 1.3% , Sorghum (Red) (*Sorghum bicolor*)- 1.2%, Pearl millet (Gundu) (*Pennisetum glaucum*)- 1.0%, Pearl millet (Naatu,) (*Pennisetum glaucum*)- 0.8%. Among the raw samples Foxtail millet (*Setaria italica*) had the highest beta-glucan content.

The soaked samples contained Kodo millet (*Paspalum scrobiculatum*)- 5.0%, Foxtail millet (*Setaria italica*) – 4.8%, Finger millet (*Eleusine coracana*)- 3.5%, Proso millet (*Panicum milliaceum*)- 2.2%, Little millet (*Panicum sumatrense*)- 2.2%, Banyard millet (*Echinochloa esculenta*)-2.1%, Sorghum(White) (*Sorghum bicolor*)- 1.7% , Sorghum (Red) (*Sorghum bicolor*)- 1.6%, Pearl millet (Gundu) (*Pennisetum glaucum*)- 1.4%, Pearl millet (Naatu,) (*Pennisetum glaucum*)- 1.2%. In this method of processing Kodo millet (*Paspalum scrobiculatum*) has the highest content of beta-glucan.

The sprouted sample contained Foxtail millet (*Setaria italica*) – 6.5%, Finger millet (*Eleusine coracana*)- 4.5%, Proso millet (*Panicum milliaceum*)- 2.7%, Sorghum(Red) (*Sorghum bicolor*)- 2.4% , Sorghum (White) (*Sorghum bicolor*)- 2.3%, Pearl millet (Gundu) (*Pennisetum glaucum*)- 2.1%, Pearl millet (Naatu,) (*Pennisetum glaucum*)- 1.7%. Here Foxtail millet (*Setaria italic*a) was recorded to be highest in beta-glucan content.

The pressure cooked sample contained Foxtail millet (*Setaria italica*) – 7.0%, Kodo millet (*Paspalumscrobiculatum*)- 6.5%, Finger millet (*Eleusine coracana*)- 5.2%, Banyard millet (*Echinochloa esculenta*)- 4.4%, Proso millet (*Panicum milliaceum*)- 3.3%, Little millet (*Panicum sumatrense*)- 3.1%, Sorghum(Red) (*Sorghum bicolor*)- 2.8% , Pearl millet (Gundu) (*Pennisetum glaucum*)- 2.7% Sorghum (White) (*Sorghum bicolor*)- 2.6%, , Pearl millet (Naatu,) (*Pennisetum glaucum*)- 2.5%. In this method of processing Foxtail millet (*Setaria italica*) was found to have the highest beta-glucan content.

Therefore Foxtail millet (*Setaria italica*) was found to have the significantly highest beta glucan content among the millets on an average of all the processing method followed. And processing of millets did have an impact on the beta-glucan levels. Pressure cooking method of processing yielded the maximum amount of beta-glucan.

CONCLUSION:

As we are in the modern and technological era our lives have become much more easier and simpler and we get everything by the touch of a button. But these benefits come along with its own adverse effects which affects our health.

And this can be mainly addressed to our drastic lifestyle and dietary modification. These factors lead to other implications such as lifestyle disorders like obesity, diabetes mellitus, non communicable diseases such as cardiovascular disorders, Dyslipidemia .

We can combat these health issues by following a healthy balanced diet which is especially rich in fibre by including whole grains, cereals, fruits and vegetables. Millets are one such super food that renders a lot health benefits like being rich in fibre, B-complex vitamins and antioxidants.

Millets also contain beta-glucan , a vital soluble dietary fibre which makes it more nutritious and beneficial as they can prevent gastrointestinal problems, diabetes, cancers, immune related diseases since they capable of increasing the immunoglobins and killer T-cells. And millets are on par or even better in the composition of beta-glucan when compared to oats.

And this positive effect on health is enhanced with respect to method of processing used. Thus to conclude, the best processing method to increase the beta glucan content in millets is by pressure cooking.

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APPENDIX – I

ETHICAL CLEARANCE

Avinashilingam

Institute for Home Science and Higher Education for Women

Deemed to be University Under category 'A' By MHRD, (Estd. u/s 3 of UGC Act 1956)

Re Accredited with 'A' Grade By NAAC, Recognised by UGC Under Section 12 B

Coimbatore - 641043, Tamil Nadu, India

Seek and Ye Shall Find

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Principal, PSG Institute of Medical Sciences & Research, Coimbatore

Member Secretary
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Department of Food Service Management & Dietetics

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Mr. K.Arulmoli (Legal Expert)
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Dr.Subhashini K. Sripathi
Dr.A. Saraswathy
Ms.D.Kavitha
Dr.S. Muthulakshmi
Dr.G.Victoria Naomi
Dr. Judith Justin
Dr.Anitha Subash

24 January 2019

To
Ms. A. Amreen Fathima
Department of Food Service Management and Dietetics
Avinashilingam Institute for Home Science and
Higher Education for Women
Coimbatore – 641 043

Dear A. Amreen Fathima,

Ref: Your proposal No. IHEC /18-19/FSMD /04 entitled "Estimation and Profiling of Beta Glucan in Selected Millets" submitted for approval to the IHEC on 30.09.18.

The study entitled "Estimation and Profiling of Beta Glucan in Selected Millets" does not come under the purview of Institutional Human Ethics Committee as no human subjects are involved. If in case any human subjects are involved in the study hereafter you are requested to re-submit.

We wish you all the best in your research endeavours.

Regards,

S. Uma Mageshwari
Dr.S.UmaMageshwari
Member Secretary

Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore ★ Institutional Human Ethics Committee