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Extraction and Fractionation of Insoluble Fibers from Foxtail Millet (*Setaria italica* (L.) P. Beauv)

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ABSTRACT

The study was carried out to investigate the extraction and fractionation of insoluble fibers from two varieties of foxtail millets. Moreover, the glucose uptake of the purified extracts was assayed. The results showed that Neutral Detergent Insoluble Fibers (NDIF) were as high as 54.59 and 55.37% for white and the yellow foxtail millets, respectively. Moreover, while the white samples yielded 15.44 11.56 and 27.00% as the respective insoluble, soluble, total dietary fibers that of the yellow offered 97, 15.91 and 27.88% as the insoluble, soluble and total dietary fibers in that order. White foxtail millet had the highest total hemicellulose (52.8%) content followed by yellow foxtail (50.34%). Cellulose and lignin were (32.41 and 31.34%) and (2.89 and 3.07%), respectively. The pectin substances were estimated to be lowest in both samples (0.806 and 0.906%). Glucose uptake was varied from 12.43 to 98.22% and those for PIM-Y from 14.36 to 98.57% and the increase was dependent on both glucose and sample concentrations. This founding fractionation procedure suggested that these purified insoluble fibers could be incorporate as low calorie bulk ingredients in high fiber foods production to reduce calorie level and help to control blood glucose levels.

Key words: Foxtail millet, insoluble fibers, extraction, fractionation, glucose uptake

INTRODUCTION

Foxtail millet (*Setaria italica* L. Beauve) is used mainly as animal feed especially in North America and Europe. However, the consumption of millet as human diet is gaining acceptance in recent times particularly, among people seeking gluten-free alternatives and healthier diets (FAO, 1995; Jiaju and Yuzhi, 1993). Millet is commonly used for preparation of many foods and beverages, such as steamed meal, porridge, bread and beer (Brink, 2006; FAO, 1995). Due to its nutritional quality, several researchers have demonstrated that foxtail millet contains high amount of bioactive compound that possess many health benefits (Pawar and Machewad, 2006;

Ushakumari *et al.*, 2004), whereas study on foxtail millet have mainly reported on the treatment of diabetes by improving cholesterol-metabolism (Choi *et al.*, 2005).

Though previous studies have attempted to determine the soluble and insoluble fibers of foxtail millet as chemical components (Malleshi and Hadimani, 1994; Rooney *et al.*, 1982; Shaohua *et al.*, 2009), no specific research has reported on its insoluble dietary fibers isolation and/or fractionation from its seeds up to date. Several methods regarding the insoluble dietary fibers isolation and/or fractionation been developed and categorized: (1) gravimetric methods, (2) gravimetric-enzymatic methods, (3) calorimetric methods and (4) chromatographic methods (Claye *et al.*, 1996). Even though the gravimetric techniques have been employed to estimate and quantify crude fiber underestimated yields leading to adoption of newer and more accurate methods. Fractionation is one such method used to quantify fiber constituents and isolate interest fractions as well as eliminate undesirable compounds.

The techniques for fractionation of dietary fibers into their individual components are limited in number (Anderson and Ydesdale, 1980; Dreher, 1987; Furda, 1977; Graham *et al.*, 1988; Monte and Maga, 1980; Southgate, 1969, 1977). Lawther *et al.* (1995) fractionated wheat straw polysaccharides into water soluble, pectic, 80% ethanol-soluble, sodium chlorite-soluble, hemicellulosic and cellulose fractions, using essentially, gravimetric methods. The hemicellulosic material was further separated into a DMSO-soluble fraction, hemicellulose types A, B and C. Claye *et al.* (1996) reported on fraction of different fibers after studying the previous attempts. Using cold and hot water extraction, enzymatic and chemical treatment, they obtained four fractions (cellulose, hemicellulose A and B and lignin). Sun *et al.* (1996) extracted six hemicellulose fractions from wheat straw and reported their chemical composition. In fact, fractionation methods are varied and developed according to the raw materials. Therefore, there is no standard method used to fractionate the fiber components. Some approaches permitted refined separation of compounds, enabling determination of molecular structure (Mukhiddinov *et al.*, 2000; Zou *et al.*, 2010).

Fractionation of foxtail dietary fiber may be an ideal, since the technique by its scope provides unique way to quantify and isolate interest fractions with limited undesirable components. Moreover, the understanding of functionality (physicochemical effects) of the individual dietary fiber components in foxtail millet in relation to human health would depend on their isolation and quantification. However, to the best of our knowledge, no data on the extraction of insoluble dietary fibers from foxtail millet their fractionation has been reported so far. Consequently, the present study was conducted to isolate and fractionate insoluble fibers from white and yellow foxtail millets. Also and the Purified Insoluble Material (PIM) was assayed on glucose uptake.

MATERIALS AND METHODS

Source of the samples and preparation of the fiber sources: Foxtail millets (*Setaria italica* L. Beauv), white and Yellow seeds were purchased from millet research institute in DongBei (Liao Ning Province, China).

The Foxtail millets were sorted and cleaned to remove foreign materials. The cleaned seeds were washed using tap water, drained and dried in an air oven at 35°C for 1 h. The grains were milled and sieved through a 100 mesh to obtain flour. Parts of the flours (500 g) were defatted overnight with n-hexane (w/v) according the ratio (1:5) at room temperature (25°C). The defatted materials were dried in an air oven at 25°C for 5 min and before being packed into polyethylene bags and stored in refrigerator at 4°C until used for study. The amyloglucosidase (Code: NS-22035) an

trypsin (Code: HS35079010) were obtained from (Novozymes Co.Ltd, China). All chemicals used in this experiment were of analytical grade.

Chemical composition of fiber sources: The defatted flours were processed for analysis of protein, moisture, ash and Neutral Detergent Fiber (NDF) using the methods previously described by James (1995). Glucose content was estimated by the method of the glucose assay kit (Megazyme K-GLUC, Wicklow, Ireland). Reducing sugar, total carbohydrate and starch were analyzed with the methods cited by Sadasivam and Manickam (1992).

Insoluble, soluble and total dietary fibers: According to the method of Prosky *et al.* (1988), 1 g of defatted flours (triplicate) were subjected to sequential enzymatic digestion by heat stable α -amylase (95-100°C), protease and amyloglucosidase (60°C). The Insoluble Dietary Fiber (IDF) was filtered and washed with hot distilled water (70°C), 95% ethanol and acetone. The IDF are dried in vacuum at 50°C overnight. Combined solution of filtrate and hot water washings was precipitated with 95% ethanol for Soluble Dietary Fibers (SDF). Precipitates were then filtrated and residues washed with 78% ethanol, 95% ethanol and acetone, dried in vacuum at 50°C overnight. Combining the values for IDFs and SDFs put out the Total Dietary Fibers (TDFs).

Fraction procedure: Figure 1 shows the extraction and fractionation procedure described by Claye *et al.* (1996) with minor modification. Prior to the optimization of yield of components, cold and hot water extractions of the fibers were used to partially remove soluble polysaccharides and proteins before enzyme treatment. This procedure has been found to reduce these components by about 20% (Anderson and Ydesdale, 1980; Claye *et al.*, 1996). Potassium hydroxide (under nitrogen atmosphere) was used in place of the detergent technique for extracting lignocellulose. Hemicellulose A and B were extracted according to the procedure described by Monte and Maga (1980). A triplicate extraction was carried to increase sample sizes as recommended by Rasper (1981).

Removal of soluble complex carbohydrates and proteins: Using the procedure previously described by Claye *et al.* (1996) with minor changes, defatted millet flours were extracted at 20°C for 2 h using slightly alkaline water (1:10 W/V ratio, pH 7.0-7.5). The mixtures were centrifuged (300 g \times 15 min at 25°C). The supernatants were discarded and the procedure repeated three times. The residues were extracted with 0.01 M EDTA for 2 h to bind cations and solubilize more pectic substances (Furda, 1977). The mixtures were filtered and the extraction repeated twice. The residues were washed twice with 80% ethanol and three times with distilled water, lyophilized and kept for further analysis. These residues represent the non-purified insoluble materials (N-PIMs).

Enzymatic extraction of non-purified insoluble materials: According to the method of Southgate (1991), the N-PIMs were enzymatically treated. The 25 g of N-PIM of both samples were weight into two liters beakers to which 0.1 M acetate buffer (50 mL⁻¹ pH 4.8) was added. The triplicate treatments were carried out under controlled pH. Amyloglucosidase (0.15 mL⁻¹) was added, the beakers were incubated at 55°C for 3 h with continuous agitation. After cooling, the pH was adjusted to eight and trypsin (5 mg) was added. The beakers were incubated at 37°C for 18 h stirring slowly. The record mixtures were filtered washed three times with 80% ethanol, one

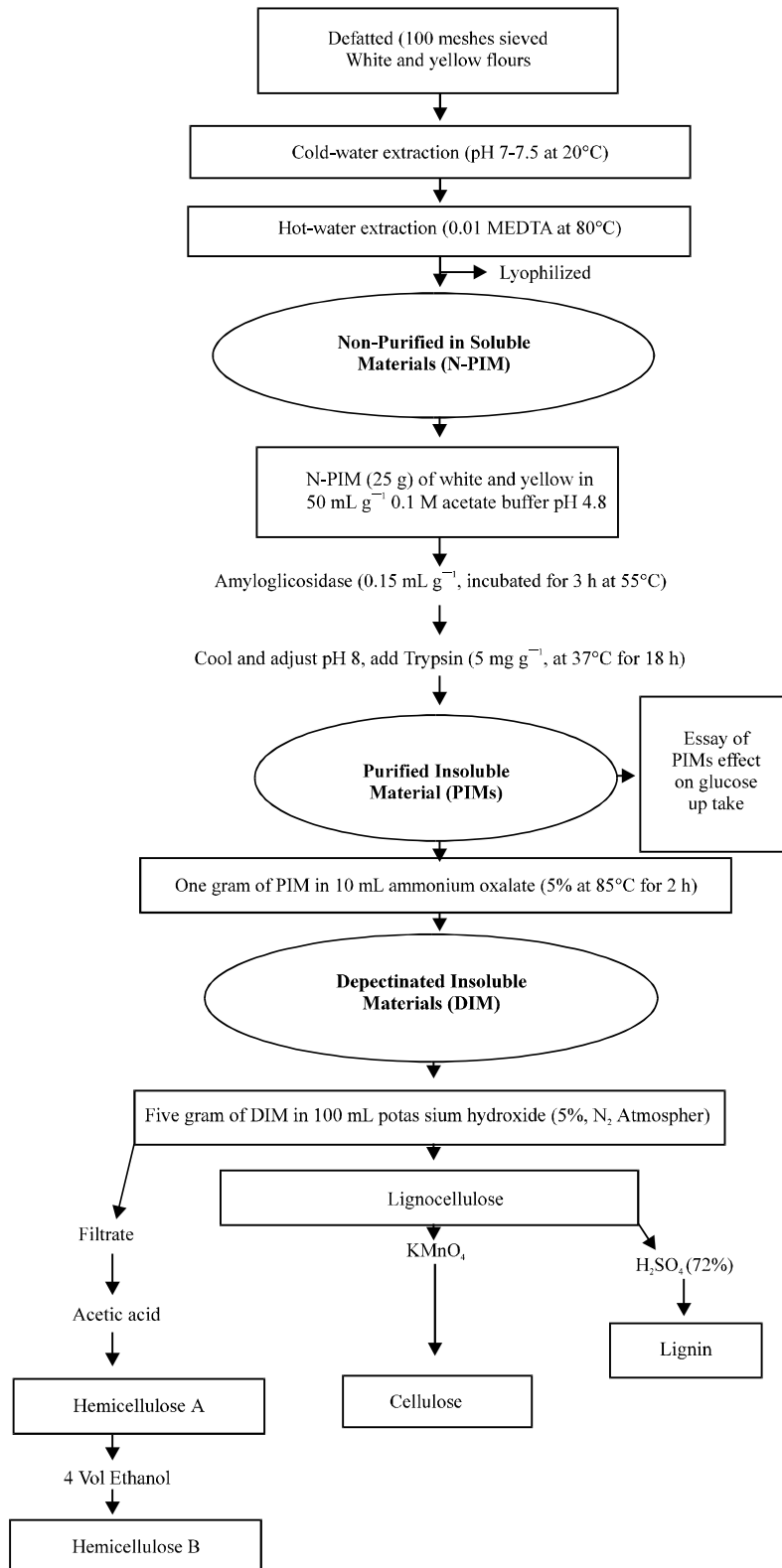


Fig. 1: Flow diagram of extraction and fractionation of *Setaria italica* insoluble dietary fibers

95% ethanol and three times of distilled water and freeze-dried. These residues represent the Purified Insoluble Material (PIM). The presence of starch in the enzyme treated fiber fractions was checked using an iodine solution.

Insoluble pectic substances: As previously described by Monte and Maga (1980), triplicate 1 g PIMs of each samples were extracted three times with 0.5% ammonium oxalate (10 mL) at 85°C for 2 h. The fiber residues were filtered and washed with ethanol, distilled water and then dried in vacuum at 35°C. These residues were called as Depectinated Insoluble Materials (DIMs). The loss in weight on drying was the yield of insoluble pectin present in the sample.

Cellulose: The method described by Robertson and Van Soest (1981) was adopted for crude cellulose determination. Briefly, 2 g of lignocellulose were extracted with 20 mL⁻¹ of combined reagent (KMnO₄/lignin buffer, ratio 2:1, v/v) in sintered glass crucible and allowed to stand for 90 min for 22°C with periodic stirring. The reagent in crucible was made to remain purple by changing frequently by duration of the extraction process. The combined reagent was drawn out by suction and the crucibles were transferred to clean pan. Demineralized solution (20 mL) was added to each crucible and allowed to stand for 5 min, refilling as necessary and then remove by suction. The completion of demineralization was indicated by removal of black manganese from white/gray cellulose.

The extraction lasted for an average of 30 min. Crude cellulose was washed with 80% ethanol, distilled water then lyophilized and store in refrigerator.

Extraction of Hemicellulose A and B: The method previously reported by Monte and Maga (1980) was adopted to extract hemicellulose A and B. Briefly, 5 g of kepectinated knsoluble Material (DIM) were weighed (triplicate) into 250 mL plastic stoppered centrifuge bottles and 100 mL of (5%, w/v) potassium hydroxide was added. The bottles were flushed with nitrogen and shaken for 24 h, then centrifuged (1500 g×10 min at 37°C); the supernatant was decanted, saved for analysis. After extracting two times, these residues could be lignocellulose. Lignocellulose was dried and at 35°C overnight and kept for further analysis. The filtrate was combined by adding 5% acetic acid and pH was adjusted to 5-5.5 and then centrifuged (4000 g×15 min). The hemicellulose A (HC-A) fraction was washed and freeze-dried. Collected supernatant from HC-A was diluted with four volumes of 95% ethanol to precipitate hemicellulose B (HC-B) fraction.

Lignin: The Klason lignin method Robertson and Van Soest (1981) was performed for the extraction of the lignin. Triplicate samples of lignocellulose (5 g) were extracted with cold 72% sulfuric acid solution (1 g, w/v) at 4°C for 30 h. Cold distilled water was added (150 mL) and the residue allowed to precipitate. The residue was washed with warm distilled water until no acid was detectable. The Crude Lignin Material (CLM) was then air dried and kept in refrigerator for further studies.

Assay of PIMs effect on glucose uptake: In accordance with the previous method described by Cirillo (1962), the commercial yeast for bakery was washed repeatedly with distilled water and centrifuged (3000×g; 5 min at 25-37°C) until the supernatant was clear and 10% (v/v) of suspension was prepared in distilled water. The amount of PIMs (1-5 mg), were added to 1 mL at different concentration of glucose solution (5-25 mmolL⁻¹) and incubated for 10 min at 37°C. The

suspension of yeast (100 μL^{-1}) was added and mixed well before incubation at 37°C for 1 h. After incubation, the tubes were cooled at room temperature (25-37°C) and centrifuged (2500 \times g, 5 min) and glucose was measured in the supernatant (Cirillo, 1962). The increased of glucose uptake by yeast was estimated using the following Eq. 1:

$$\text{Activity (\%)} = \frac{\text{Abs. control} - \text{Abs sample}}{\text{Abs. control}} \times 100 \quad (1)$$

Where:

Abs. control: is the absorbance of the control reaction (containing all reagents except the test sample),

Abs. sample: is the absorbance of the test sample.

Statistical analysis: One-way analysis of variance (ANOVA) was carried out on each of the variables and the Least Significant Difference (LSD) test at α level of 0.05 was performed using SAS software (SAS 8.1 for Windows, SAS Inc., Cary, NC, USA) to compare the difference between treatment means. Results were expressed as the Mean \pm standard deviation of three separate determinations.

RESULTS AND DISCUSSION

Chemical composition of fiber sources: The chemical composition of both defatted fiber sources is presented in Table 1. The values of the crude protein varied from 12.02% to 13.81%. Significant difference ($p < 0.05$) was found between the two determinations and these results were slightly comparable to those reported by Wankhede *et al.* (1979). For the moisture content no significant difference ($p < 0.05$) was observed in both white and yellow fiber sources. This was in a good agreement to that reported on moisture in foxtail millet by Shaohua *et al.* (2009). The ash values (0.79-0.87%), respectively were found to be low than that reported in previous study, this variation depends of the agricultural and/or cultivation conditions (NAS, 1996). Total starch, carbohydrate and reducing sugar were significantly different ($p < 0.05$) in both the fiber source samples. Results for reducing sugar (115.72-17.33 mg g^{-1}) fell slightly comparing to those reported by Raghavendra

Table 1: Chemical composition of defatted foxtail millets^a

Nutrient	Defatted foxtail millet	
	White	Yellow
Protein (%)	13.81 \pm 0.04 ^b	12.02 \pm 0.08 ^c
Moisture (%)	8.25 \pm 0.16 ^{bc}	8.44 \pm 0.26 ^{bc}
Ash (%)	0.79 \pm 0.11 ^{bc}	0.87 \pm 0.06 ^{bc}
Starch (mg g^{-1})	57.57 \pm 0.30 ^b	52.44 \pm 0.29 ^c
Carbohydrate (mg g^{-1})	67.68 \pm 0.65 ^b	53.26 \pm 0.29 ^c
Reducing sugar (mg g^{-1})	115.72 \pm 0.14 ^c	117.33 \pm 0.21 ^b
Glucose (mg g^{-1})	70.67 \pm 0.51 ^c	72.20 \pm 0.51 ^b
Neutral detergent fiber (%)	54.59 \pm 0.30 ^c	55.37 \pm 0.09 ^{ba}

^aMeans of triplicates, values in the same rows with different letters are significantly different ($p < 0.05$). Standard curves and R^2 of different determinations were: Starch: $y = 0.699x + 0.081$, $R^2 = 0.9924$. Carbohydrate: $y = 0.853x + 0.009$, $R^2 = 0.9979$. Reducing sugar: $y = 0.706x - 0.102$, $R^2 = 0.9914$. Glucose: $y = 0.1964x - 0.0948$, $R^2 = 0.9989$

Rao *et al.* (2011). This variation could be due to the factors such as agricultural conditions as reported by NAS 1996. Perhaps, it could also be related to milling-separation procedures and/or varietal/environmental conditions of the fiber source samples. The total starch and carbohydrate values (57.57-52.44 and 53.26-67.68 mg g⁻¹), respectively were high and close to those reported by Wankhede *et al.* (1979). Glucose content in the both samples were estimated to be high (70.67 and 72.20 mg g⁻¹) and significantly different (p<0.05). The NDF content in both fiber source samples were significantly different (p<0.05) and seated within the ranges of those reported by Aniola *et al.* (2009) and Smith and Kallenbach (2006).

Insoluble, soluble and total dietary fibers: The high values of fiber were found in the both defatted samples and no significant difference (p<0.05) was recorded between them Table 2. However, high value of NDF was found in yellow seed and significantly different (p<0.05) compared to the values obtained for white seed Table 1. Anju and Sarita (2010) reported on foxtail millet flour for IDF (15.88%), SDF (11.04%) and TDF (26.92%). These values are similar to those reported in this investigation, but a slight difference was observed due to the quality of the flour; perhaps contained amount of other components (i.e, insoluble polysaccharides) and could not be easily hydrolyzed by enzymes.

Insoluble pectic substances: The pectic substances play an important role in plant life, their primary function is the cementing together of the individual cells that compose the plant.

The insoluble pectins removed for white (0.806%) and yellow (0.906%) foxtail millet fiber fractions were presented in Table 3 and significantly different (p<0.05) to each other. As schematically shown in Fig. 1, the pure insoluble materials were obtained after removing the pectin substances. In our study, the lowest values have been found when compared to those reported by Claye *et al.* (1996) where ammonium oxalate utilization led to the complete extraction of pectin from the various fiber sources and Lawther *et al.* (1995) when fractionated wheat straw. These founding results are not in good agreement to those reported during our investigation.

Cellulose: The major component in the rigid cell walls in plants, cellulose is a linear polysaccharide polymer with many glucose monosaccharide units. The crude cellulose content of both PIMs (white and yellow fibers) were elevated (32.41 and 31.34%), respectively in Table 3 and significantly different (p<0.05). These values were significantly higher to that reported in foxtail millet by

Table 2: Insoluble, soluble and total dietary fiber of defatted fiber sources (g/100 g)^a

Source of fiber	Insoluble	Soluble	Total
White foxtail	15.44±0.22 ^{bc}	11.56±0.47 ^{bc}	27.00±0.69 ^{bc}
Yellow foxtail	15.97±0.27 ^{bc}	11.91±0.66 ^{bc}	27.88±0.21 ^{bc}

^aMeans of triplicates, values in the same column with different letters are significantly different (p<0.05)

Table 3: Defatted fiber sources and hemicellulose, cellulose, lignin and insoluble pectin (%) in fractionated PIM^a

Source of fiber	Hemicellulose		Cellulose	Lignin	Pectin
	A	B			
White fox	19.41±0.12 ^b	33.39±0.07 ^b	32.41±0.06 ^b	2.89±0.09 ^c	0.806±0.00 ^c
Yellow fox	18.69±0.16 ^c	31.65±0.57 ^c	31.34±0.07 ^c	3.07±0.06 ^b	0.906±0.02 ^b

^aMeans of triplicates, values in the same column with different letters are significantly different (p<0.05)

Wankhede *et al.* (1979), but they did not process for crude cellulose by fractionation procedure. However, the values reported in our study were within the range to those reported by Lawther *et al.* (1995) in wheat straw polysaccharides. Claye *et al.* (1996) also brotherly reported in insoluble fiber from five fiber sources and Gaspar *et al.* (2005) reported on fractionation and utilization of corn fiber carbohydrates. Except the result given by Wankhede *et al.* (1979), no cellulose data was available for foxtail millet fiber for comparison.

Hemicellulose A and B: Hemicellulose (also a polysaccharide) consists of shorter chains sugar units as opposed to glucose molecules per polymer in cellulose. It is a branched polymer, while cellulose is unbranched. Hemicellulose A and B extracted from DIM for white foxtail and yellow foxtail millets are shown in Table 3. Hemicellulose A ranged for white fox (19.41%) and yellow fox (18.69%) were significantly different ($p < 0.05$) and lower than that reported by Wankhede *et al.* (1979) in setaria, while hemicellulose B were (33.39 and 31.65%), respectively for the both white and yellow *Setaria italica* and lower than founding results.

These differences may be due to the procedures used to the pretreatment of setaria seeds and/or perhaps to the method used for extracting the HC-A and HC-B. In addition, the results reported by Wankhede *et al.* (1979) were most important for comparison in our research, even though they did not provide their results using fractionation procedures. Nevertheless, other searchers reported on HC-A and HC-B when fractionated insoluble fiber from five sources (Claye *et al.*, 1996). Lawther *et al.* (1995) fractionated wheat straw and Gaspar *et al.* (2005) studied fractionation and utilization of corn fiber carbohydrates. The results given in our study are within the range provided by above statements. Now-a-days, no reports found on HC-A and HC-B extraction in *Setaria italica* for comparison, except those given by Wankhede *et al.* (1979).

Lignin: Crude lignins content in lignocellulose from both extracted fiber sources are shown in Table 3. Significant difference ($p < 0.05$) was found between white (2.89%) and yellow (3.07%). An appropriate length of time for acid hydrolysis is still controversial. Wankhede *et al.* (1979) brotherly reported crude lignin value of 0.04% for *Setaria italica*. This value as well as others in the literature is not consistent, probably because of differences in methodology and length of time of hydrolysis, whereas, other studies reported (14.13-25.83%) in wheat straw (Lawther *et al.*, 1995) and (12.2%) in corn (Gaspar *et al.*, 2005). Moreover, these differences may be due to some carbohydrates monomer loss during the fractionation.

Assay effect on PIMs on glucose uptake: The effects of PIM-W and PIM-Y on the rate of glucose uptake cross-cell membrane are mostly studied an *in vitro* system comprising yeast cell suspended in glucose solution in various concentrations (5-25 mmol L⁻¹) (Fig 2). This study was a simple assay after purification of the extracted insoluble (white and yellow setaria italica) fibers by fractionation procedure. The amount of glucose remaining in the medium after specific time serves as indicator of glucose uptake by yeast cells.

Results from our study showed that PIM-W and PIM-Y increased significantly ($p < 0.05$) different of the glucose uptake in yeast cells. The values for PIM-W were varied from 12.43 to 98.22% and those for PIM-Y from 14.36 to 98.57% and the increase was dependent on both glucose and sample concentrations. However, the percentage increased in glucose uptake by yeast cells was inversely proportional to glucose concentration and found to decrease with increase in the molar concentration of the glucose solution. This was in good agreement to those reported by Ahmed and

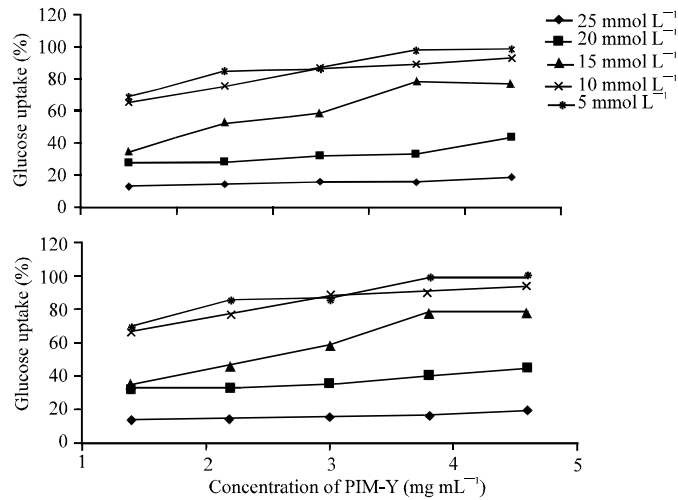


Fig. 2: Effect of PIM-W and PIM-Y on glucose by yeast uptake by yeast cells at different glucose concentrations

Urooj (2010) when they studied *in vitro* hypoglycemic potential of *Ficus racemosa* Stem bark. This mechanism suggested that glucose transportation across the yeast cells membrane could be more attractive form of an *in vitro* screening method for hypoglycemic effect of various compound/medicinal plants (Teusink *et al.*, 1998). In addition, this research suggests and reports the characteristics of glucose transport in the presence/absence of PIMs from *Setaria italica* and projected abilities of glucose transport across the yeast cell membrane function of external glucose concentration as well as sample concentration. This relationship will cease to set out the yeast cells reach saturation point (Ahmed and Urooj, 2010).

CONCLUSION

The present investigation demonstrated the advantage of fractionation procedure and revealed that the major component of *Setaria italica* fiber-fractions could be fractionated and therefore, provides information for further research such as physiological effects of the glucose uptake that we processed by assay. This point needs to be completed by glucose-transport across yeast cell membrane in further study. The amount of fiber components in our study showed slight difference between fiber sources. Insoluble fibers have been implicated to bind bile acids and reduce availability of minerals. A good proportion of hemicellulose, cellulose and glucose up-take of both fiber sources could be food ingredient (bulk agent) in food product formulations which could be satisfactory for the market demand of high-fiber foods and will increase more supporting data become available on their disease prevention ability.

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