

Insoluble Vegetable Fiber as a Potential Functional Ingredient: *In vitro* Studies on Hypoglycemic and Hypocholesterolemic Effect

Dhiraj D. Gohil, S. S. Lele*

Food Engineering and Technology Department, Institute of Chemical Technology, Matunga, Mumbai, India

*Corresponding author: dr.smita.lele@gmail.com

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Abstract Vegetable and fruit derived fibre plays a crucial role in promoting good health. Soluble and insoluble fiber has different physiological roles in human body. Insoluble fiber is known for having several beneficial effects like anti-constipating, hypoglycemic, hypocholesterolemic. In the present study, insoluble dietary fiber from vegetables viz., *Nelumbo nucifera*, *Murraya koenigii*, *Abelmoschus esculentus* and *Brassica oleracea* were investigated *in vitro* for their hypoglycemic and hypocholesterolemic effect. The hypoglycemic effect was studied by measuring glucose adsorption, glucose diffusion and amylase inhibitory activity whereas hypocholesterolemic effect was observed by bile salt (Sodium cholate) adsorption study. Results indicate that *N. nucifera* exerts significant effect on glucose adsorption (7.40 ± 0.14 mmol/g) and amylase inhibitory activity (13.19 ± 0.66 %). Similarly glucose diffusion studies showed low diffusion values in case of *N. nucifera* as compared to others up to an hour. Also, *N. nucifera* showed highest sodium cholate binding capacity (10.11 ± 0.20 %) compared to other fibres when cellulose was used as a control. Thus, this study suggests that insoluble fibre from *N. nucifera* can be used for fortification of functional food products aimed at controlling glucose and cholesterol level.

Keywords: hypoglycemic, hypocholesterolemic, vegetables insoluble fiber

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1. Introduction

Dietary fiber is the edible part of plant or an analogous carbohydrate that is resistant to digestion in intestine [1]. Based on water solubility, dietary fiber is grouped into two categories namely soluble dietary fiber which is well fermented by human colonic bacteria and insoluble dietary fiber that is poorly fermented [2]. High fiber diet reduces the risk of many diseases like cancers, cardio-vascular disease, obesity, gastrointestinal disorders and diabetes [3,4,5,6].

The hypoglycemic and hypocholesterolemic potential of dietary fibers in managing diabetes and cardiovascular disease is very well documented [3,5,7]. In various studies, hypoglycemic effect of dietary fibers has been investigated [8,9,10,11,12,15]. The mechanism by which dietary fibre exerts hypoglycemic effect includes inhibiting amylase activity, glucose adsorption and glucose diffusion [10,11]. Apart from hypoglycemic effect, dietary fibre also exerts hypocholesterolemic effect by binding bile salts leading to their increased fecal excretion [16,17,18]. This in turn promotes utilization of endogenous cholesterol in bile salt synthesis leading to an overall hypocholesterolemic effect [12,13].

The hypoglycemic and hypocholesterolemic effect of dietary fibres has been investigated in studies wherein the fibre was used in the form of isolated constituents, fiber-rich foodstuffs or fiber fractions. However, the role of insoluble fibers from whole vegetable remains unexplored. Furthermore, soluble fibre undergoes fermentation leading to production of short chain fatty acid [19] whereas most of the insoluble dietary fibres are excreted. The insoluble fiber is very well known as an anti-constipation agent through its water holding capacity is very well known. Thus, the present study was undertaken to explore the health promoting effect of insoluble fibre from locally available vegetables viz. *Nelumbo nucifera* Stem (Lotus stem), *Murraya koenigii* leaves (Curry leaves), *Abelmoschus esculentus* var. *Pusa Sawani* (Okra) and *Brassica oleracea* var. *capitata* (Green Cabbage) by determining the capabilities of these fibers in lowering serum glucose and cholesterol level.

2. Materials and methods

2.1. Materials

All vegetables were procured from local Mumbai market. Sodium cholate was purchased from Sigma-Aldrich (Mumbai, India) and all other chemicals and

enzymes were obtained from HiMedia laboratories (Mumbai, India). The chemicals were all of analytical grade. Heat stable alpha-amylase, amyloglucosidase were gifted by Genecore(Mumbai, India). Glucose Assay kit (Eco-pak kit, GOD-POD) was purchased from Accurex Biomedical Pvt. Ltd. (Mumbai, India).

2.2. Extraction of Insoluble Dietary Fibers

Vegetables procured from the local market were cleaned and dried at 60°C. The extraction of vegetable insoluble fibers was done by *enzymatic extraction* using standard Association of Official Analytical Chemists (AOAC 1990) method. The defatted vegetable powder was digested with heat stable alpha-amylase (100°C, 1 h) and then digested with protease (60°C, 1 h), followed by incubation with amyloglucosidase (60°C, 1 h) to remove protein and starch. The enzyme treated mixture was centrifuged (5000 rpm for 20 min at 25°C). The residue recovered (IDF) was washed thrice with water, 70% ethanol, absolute ethanol and acetone sequentially. The residue was oven-dried (60°C, Overnight) and stored for further analysis.

2.3. Estimation of Glucose Adsorption Capacity

The glucose-adsorption capacity was determined in accordance with method mentioned in [11]. 1 g of fibers and cellulose (Alphacel-Nonnutritive fiber, ICN Nutritional Biochemicals, Cleveland, OH) was mixed with 100 ml of glucose solution, concentration ranging from 5-50 mmole/l. The mixture was stirred, held in a water bath at 37°C for 6 h, followed by centrifugation at 3500g for 15 min. At the end of adsorption, the glucose content in the supernatant was measured using the Glucose assay kit to estimate the amount of glucose adsorbed on the fibers.

2.4. Estimation of Glucose Diffusion and Glucose Dialysis Retardation Index (GDRI)

Glucose dialysis retardation index was determined as given in [8] with slight modifications. A solution was prepared by mixing 0.1 gm of fiber sample in 5 ml of glucose solution (10mmole/l) and was dialyzed against 40 ml of distilled water at 37°C using a dialysis membrane with a molecular weight cut off value of 12,000 D. After incubation of 20, 30, 60, 120 and 180 min the glucose content in the dialysate was measured by Glucose Assay kit. A control test was also prepared without addition of fiber.

The GDRI was calculated using following equation (1):

$$GDRI = 100 - \left[\left(\frac{\text{Glucose content in dialysate with fiber addition}}{\text{Glucose content in dialysate with no fiber addition}} \right) \times 100 \right] \quad (1)$$

2.5. Analysis of Alpha-Amylase Activity

The effect of fiber on glucose production rate and amylase inhibitory activity was determined as per [8] with slight modifications. An incubation mixture containing 0.25 gm of fiber sample and 1 gm of alpha-amylase in 10 ml of potato starch solution (4 gm/100 ml) was incubated at 37°C. After 60 min, starch digestion was stopped by addition of absolute ethanol (15 ml). Then it was

centrifuged at 3500 rpm for 15 min and the supernatant was analyzed for glucose content to obtain the glucose production rate ($\mu\text{mole per hour}$) by Glucose Assay kit. A control test was also done without addition of fiber. The amylase inhibitory activity (%) was defined as the percent decrease in the glucose production rate over the control.

2.6. Quantification of Sodium Cholate Binding

The binding experiment was performed using methodology as suggested by [20] with some modifications. Sodium salt of cholic acid, a primary bile acid was used in the study. Two grams of dietary fibers were incubated with 10 mmole/l sodium cholate in 100mL of phosphate buffer (pH 7.0). The mixture was agitated 120 rpm for 3 h at 37°C. At the end of adsorption, 2 mL of the sample was collected, and absolute ethanol (8 mL) was added. After centrifugation (4000g for 20 min) the concentration of cholate in the supernatant was determined by the colorimetric assay, where 50- μl samples were pipetted into 2-ml polypropylene tubes before adding 800 μl of concentrated sulfuric acid. Tubes were vortexed and allowed to cool to room temperature, and absorbance was determined at 389.

2.7. Statistical Analysis

All analysis were done in triplicate and are expressed as mean value \pm S.D. The statistical significance was assessed by one way analysis of variance followed by Tukey's test for the determination of level of significance using GraphPad Prism 5.0.

3. Results and Discussion

3.1. Effect on Glucose Adsorption

A series of different concentrations of glucose (5-50mmole/l) were used to study glucose adsorption capacity of vegetable insoluble fibers. As seen in Figure 1, adsorption capacities of *N. nucifera*, *A. esculentus* and *B. oleracea* (0.03-7.40 mmole/g) were significantly higher ($p < 0.01$) compared to that of *M. koenigii* and cellulose (0.08-4.72 mmole/g) at a different glucose concentration. At high glucose concentration (50mmole/l), adsorption capacity of *N. nucifera* and *A. esculentus* was significantly ($p < 0.01$) higher as compared to others and cellulose. When low glucose concentration used (5mmole/l), the glucose-adsorption capacity of fibers was found to be 0.03-0.69 mmole/g. At 5mmol/l glucose concentration, *N. nucifera* (0.69 \pm 19 mmole/g) has the highest adsorption capacity. Thus, the results indicated that the fibers could effectively bind to glucose even at low concentrations of glucose thereby reducing the amount of accessible glucose in small intestine. It is observed that the glucose adsorption capacity of fibers is directly related to available glucose concentration. Similar observations were reported by Chau et al [15] and Ou et al [11] for insoluble fiber-rich fractions from *Averrhoacarambola* and wheat bran for ability to bind glucose and further decrease available glucose. Thus the vegetable insoluble fiber may be beneficial with respect to reducing amount of accessible glucose in the small intestine.

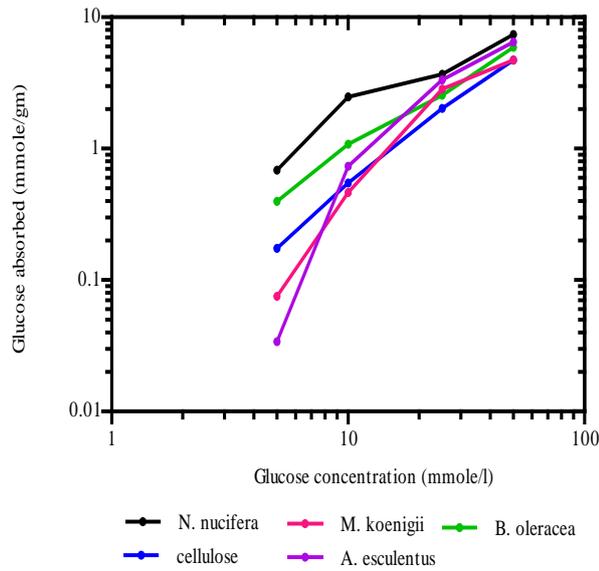


Figure 1. Log graph of Glucose absorbed vs. Glucose concentration

3.2. Determination of Glucose Diffusion

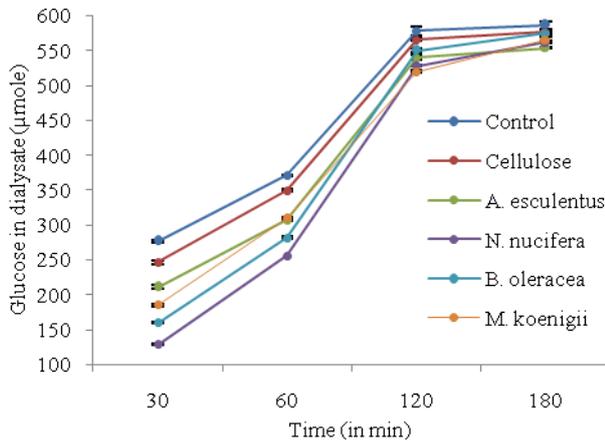


Figure 2. Effect of insoluble fibers on Glucose diffusion with respect to time

In this study, effect of vegetable insoluble fibers on glucose diffusion was compared with that of control from 30 min to 180 min. It was observed that, all the fibers significantly ($p < 0.01$) decreased diffusion of glucose across dialysis membrane as compared to control as seen in Figure 2. The glucose contents in the dialysate among the vegetable fibers and cellulose ranged from $128.82 \pm 1.06 - 246.85 \pm 2.75 \mu\text{mol}$ (at 30 min) to $553.72 \pm 0.61 - 576.72 \pm 0.93 \mu\text{mol}$ (at 180 min). Glucose diffusion was lowest in *N. nucifera* at 30 and 60 min (128.82 ± 1.06 and $256.21 \pm 0.70 \mu\text{mol}$) as compared to control and other fibers. After incubation for 180 min, the glucose content in dialysate was reduced by all the fibers tested as compared to that of control ($586.26 \pm 5.71 \mu\text{mol}$) however reduction was highest in *A. esculentus* ($553.72 \pm 0.61 \mu\text{mol}$) and lowest in cellulose ($576.72 \pm 0.93 \mu\text{mol}$). Maximum glucose diffusion decreased in the order control > cellulose > *B. oleracea* > *M. koenigii* > *N. nucifera* > *A. esculentus*. Chau et al [15] suggested that the delay in diffusion could possibly attributed to their glucose adsorption capacities and complex meshwork of fibers which makes glucose unavailable for diffusion [5]. The above result revealed that the all insoluble fiber efficiently decrease the glucose

diffusion across dialysis membrane with different degree of diffusion depending on vegetable source, thereby controlling glucose level.

3.3. Effect of Fibers on GDRI

Glucose dialysis retardation index (GDRI) is a useful *in vitro* index to predict the effect of a fiber on the delay in glucose absorption in the gastrointestinal tract [6,11]. Effects of insoluble fibers on GDRI with respect to time are reported in Table 1. GDRI maximum value was reached after 30 min for all fiber tested and values were reduced as time increases. At 30 min, the GDRI of the all vegetable insoluble fibers (23.82-53.46%) were significantly ($P < 0.05$) higher than that of cellulose (10.81%). Similar trend was observed at 60, 120 and 180 min of incubation. Further, it was observed that *N. nucifera* fibers have the higher GDRI values amongst other studied fibers at 30 min (53.46%) till 60 min (30.98%). Based on above results, an insoluble fiber from different sources showing high retardation index and possibly helps in showing hypoglycemic effect.

Table 1. Glucose dialysis retardation index

Insoluble Fiber	GDRI			
	30 min	60 min	120 min	180 min
Cellulose	10.81±0.99 ^a	5.81±0.53 ^f	2.08±0.37 ^j	0.95±0.16 ⁿ
<i>A. esculentus</i>	23.82±0.92 ^b	17.21±0.34 ^g	6.62±0.64 ^l	4.90±0.10 ^o
<i>N. nucifera</i>	53.46±0.38 ^c	30.98±0.19 ^h	8.59±0.18 ^{km}	3.50±0.42 ^{po}
<i>B. oleracea</i>	42.13±0.64 ^d	24.07±0.62 ⁱ	4.86±0.43 ^l	1.27±0.34 ^p
<i>M. koenigii</i>	33.01±0.77 ^e	16.34±0.41 ^g	10.07±0.28 ^m	2.25±0.90 ^o

a-m, values in the same column with same superscript are not significantly different at $p < 0.001$

n-p, values in the same column with same superscript are significantly different at $p < 0.01$

3.4. Effect of Fibers on α -amylase Activity

Effect of fibers on α -amylase activity is presented in terms of glucose production ($\mu\text{g/h/g}$ of fiber) and enzyme inhibitory activity (in %) is shown in Table 2 and Figure 3. Activity of α -amylase was influenced significantly by insoluble fibers. The glucose production rate was lower in all fiber than that of control. The amount of glucose produced in test for *N. nucifera* ($672.59 \pm 5.13 \mu\text{g/h/g}$) was significantly ($P < 0.01$) lower than that of control ($774.81 \pm 6.79 \mu\text{g/h}$). The reduction in glucose production can also be interpreted in terms of increase in enzyme inhibitory activity. The Table 4 revealed that *N. nucifera* fiber could exhibit a stronger effect in reducing the activity of alpha-amylase (by 13.19%) as compared to those of cellulose (9.94%), *B. oleracea* (8.80%), *A. esculentus* (7.84%) and *M. koenigii* (6.50%). These result revealed that vegetable fiber could exhibit an effect in decreasing amylase activity, with *N. nucifera* having the highest inhibitory activity. The variations in the inhibitory activity among the studied fibers showed that the inhibition depended on the source of vegetable fiber. This effect of insoluble fibers might be due to several possible factors such as fiber concentration, presence of inhibitor on fibers, capsulation of starch & enzyme by fibers and

direct adsorption of enzyme on fibers, thereby reduces accessibility of the starch leading to the decrease in amylase activity [8,15]. The differential in reduction of enzyme activity could be due to compositional difference in insoluble fiber from different sources.

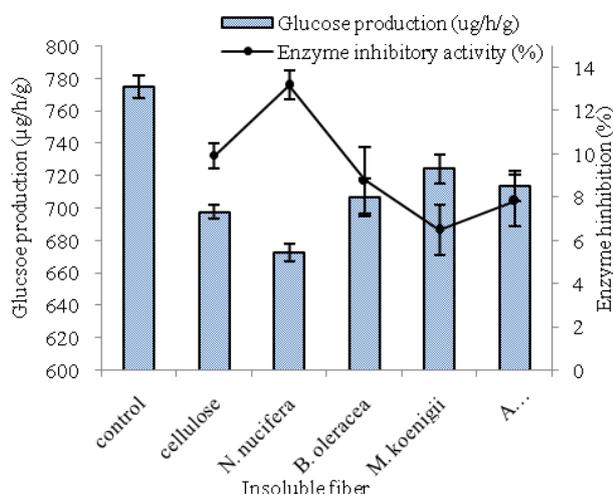


Figure 3. Effect of various insoluble fibers on α -amylase activity

3.5. Binding Capacity for Sodium Cholate

The Percentage of sodium cholate binding to vegetable insoluble fibers is shown in Table 4 and results are also expressed as sodium cholate binding in terms of mmole per gm fiber in Table 2 and Figure 4. All tested insoluble fibers were capable to interact with sodium cholate with different degree of binding. The percentage of sodium cholate bound to fiber ranges from 1.17-10.11% (5.82 - 50.55 mmole/g). Among these fibers, *N. nucifera* was most effective, showed significantly ($P < 0.01$) highest

binding capacity of 10.11% (50.55 ± 0.10 mmole/g) for sodium cholate. Further, *A. esculentus*, *B. oleracea* and *M. koenigii* showed comparatively low absorption of sodium cholate, 8.34, 8.23 and 8.06% (41.71 ± 1.38 , 41.18 ± 3.02 and 40.26 ± 0.91 mmole/g) respectively and lowest binding was observed with cellulose of 1.17% (5.82 ± 1.60 mmole/g). Binding is a result of hydrophobic interaction between insoluble fiber and sodium cholate [22]. Dongowski G. [21] showed that dietary fiber preparations from a large group of cereals, fruits and vegetables were able to bind bile acids *in vitro*. In one of the recent study, Hatim et al [23] reported that the binding capacity of synthetic fibers for bile salt were very low. Wang et al [17] showed binding capacities of insoluble fibers from various seaweeds (7-27 mmol/g) which is comparatively lower than vegetable fibers investigated in the present study (40.26-50.55 mmol/g). This further indicates that the studied vegetable fiber has good hypocholesterolemic effect.

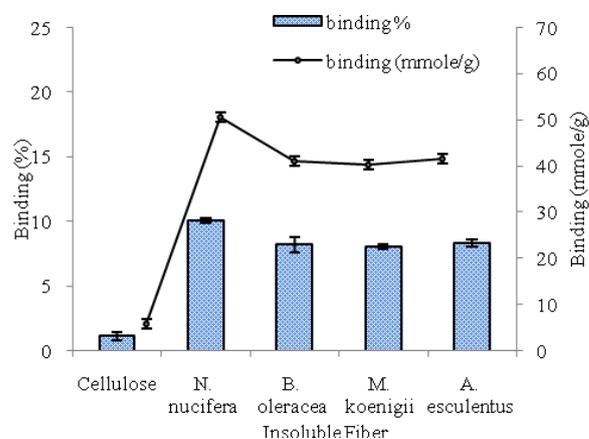


Figure 4. Sodium cholate binding capacity of insoluble fibers

Table 2. Effects of fibers on α -amylase activity and sodium cholate binding capacity

Fibers	Amylase activity		Sodium Cholate Binding capacity	
	Glucose production (µg/h/g)	Enzyme inhibitory Activity (%)	Binding (in mmole/g)	Binding (in %)
No fibers	774.81±6.79 ^a	-	-	-
Cellulose	697.78±4.44 ^b	9.94±0.57 ^d	5.82±1.60 ^f	1.17±0.32 ^f
<i>Nelumbo nucifera</i>	672.59±5.13 ^c	13.19±0.66 ^e	50.55±0.10 ^e	10.11±0.20 ^e
<i>Brassica oleracea</i>	706.67±11.76 ^b	8.80±1.52 ^d	41.18±3.02 ^h	8.24±0.60 ^h
<i>Murraya koenigii</i>	724.44±8.89 ^b	6.50±1.15 ^d	40.26±0.91 ^h	8.05±0.18 ^h
<i>Abelmoschus esculentus</i>	714.07±9.25 ^b	7.84±1.19 ^d	41.71±1.37 ^h	8.34±0.27 ^h

a-h, values in the same column with same superscript are not significantly different at $p < 0.001$

4. Conclusion

The results revealed that the studied vegetable insoluble fiber could effectively adsorb glucose, retard the glucose diffusion, inhibit the α -amylase activity and bind sodium cholate. The *N. nucifera* showed highest hypoglycemic effect followed by *B. oleracea*, *M. koenigii*, *A. esculentus*. In case of hypocholesterolemic study *N. nucifera* exhibited highest sodium cholate binding capacity and rest all of the vegetable fiber showed comparable effect. Since *N. nucifera* (lotus stem) insoluble fiber showed the most promising results, thus it can be more often included in the diet for its suggestive health promoting role.

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