An Invitro Study on Predicted Glycemic Index and Bioactive Component of Fortified - Bread Using Senna Auriculata

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ABSTRACT

Bread is an affordable staple food available world wide. People demand for the high quality breads for consumption. Bread is considered to be a daily diet food for most of the people around the world. Foods with reduced glycemic index shows less risk of some chronic diseases like diabet and stroke. Senna auriculata a plant compound which is a potential inhibitor of diabetes. Low concentration possessed high radical scavenging activity, 90% of radicals were scavenged at lower concentrations. It further proved to have the anti-hemolytic effect. The extract inhibits the alpha amylase and alpha glycosidase activity. The bread contains large amount of digestible starch which leads to glycemic index. The foods with higher glycemic index leads to type 2 diabetes mellitus. Incorporation of Senna auriculata in bread is the effective method to produce low glycemic index foods. This study was aimed to evaluate digestibility of starch and glycemic index in bread through the incorporating Senna auriculata as fortified bread sample. At 60 mins of dialysate, the concentration of resistant starch of bread sample with Senna Auriculata exhibited to be low when compared with the control. The Senna auriculata fortified bread sample is compared with the control which the regular bread based on their crust, colour, symmetry, texture, eating quality, and overall quality of the bread. Bread with 6% of extract was sensory evaluated. Bioactive components like phenolic, flavonoid found to be retained in fortified bread sample. Based on the biofunctional properties developed the evaluation is analysed at the retention time of the process. The sample has higher resistant starch and lower glycemic index. This overall invitro findings gives us a promising result that due to the lower glycemic effect the Senna auriculata fortified bread sample is situable and recommended for diabetes patients.

INTRODUCTION

Bread is an affordable staple food available world wide. People demand for the high quality breads for consumption. Using bread numerous studies have been conducted in bread making processes. Generally the breadmaking is processed using wheat flour, water, salt, sugar and yeast, mixed together in varying proportions to make a dough, which is further subjected to fermentation and baking (Sivam et al., 2010). Bread is considered to be a daily diet food for most of the people around the world. Since bread is a common component in western diet, which delivers fiber polysaccharides and antioxidants in high
concentration. Normally, functional foods which have higher antioxidant and dietary fibers are considered to be the most demandable food, because of the health associated benefits, such as cancer, cardiovascular disorder, degenerative diseases and Diabetes etc. The bread contains large amount of digestible starch which leads to glycemic index. The foods with higher glycemic index leads to type 2 diabetes mellitus \cite{Bhupathiraju et al., 2014}. Many research shows that amount and type of carbohydrate in food affects the glucose levels in the body which is the predictor of blood glucose which causes GI. To control the diabetes, the Glycemic index should be managed which is the major tool for diabetes.

\textit{Senna auriculata} is a evergreen shrub with yellow colour flowers which are grown in India. This plant is recognized to report its uses in Ayurvedic and Siddha system for treating various diseases. As it has the property to inhibit the carbohydrate digestive enzymes like alpha - amylase and alpha glucosidase \textit{Senna auriculata} can retard the starch in food. Incorporating \textit{Senna auriculata} in bread is the effective method to produce low glycemic index foods. Inspite of this inhibitory effect of \textit{Senna auriculata} on the digestive enzymes, a well know health benefit is the antioxidant capacity \cite{Aparna and Govindasamy, 2018}. The invivo oxidative stress along with associated damages can be reduced by the interactions with reactive oxygen species (ROS) \cite{Nabavi et al., 2012}. The aim of this study is to produce bread which inhibits the glycemi index, starch degradation, quality parameters, efficiency of texture, etc.

\textbf{MATERIALS AND METHODS}

\textit{Senna auriculata} (Aavaram poo), wheat flour (Carbohydrate 25%, fat 1%. gluten 11% ) and dry yeast were purchased from local stores.  

\textbf{Senna auriculata} extract preparation

The \textit{Senna auriculata} was washed, dried and ground into a fine powder. To a final mass of 300 grams, 100ml of water was added and stored for 3 - 5 days at room temperature. The extract was then centrifuged at 4000g for 15 mins. The supernatant collected was tested for further invivo activities.

\textbf{Evaluation of invivo - antioxidant activity}

Based on the protocol \cite{Brand-Williams et al., 1995} the antioxidant activity of the aqueous extract was evaluated. 0.1mM DPPH solution of was prepared using ethanol and 100 micro-liter of DPPH was added to \textit{Senna auriculata} aqueous extract solutions of different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature for 30 mins. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. DPPH alone serves as control. Gallic acid was used for comparison. The triplicates value obtained were calculated.

\textbf{HRBC membrane stabilization assay}

Based on the protocol \cite{Varghese et al., 2017}. 2 ml of blood was drawn and added to EDTA to prevent coagulation. To 100 μL of blood was added to the different concentrations of the \textit{Senna auriculata} aqueous extract. The mixture was incubated at 37°C for 30 mins. Blood with distilled water serves as control. The sample mixture was then centrifuged at 300 rpm for 10 mins. The supernatant was removed.
from each tube and placed in 96 well plates. The absorbance was measured at 517 nm using a UV-Vis spectrophotometer. The triplicates values obtained were calculated.

\[ \text{Percentage of inhibition} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \right) \times 100 \]

**HRLC-MS investigations**

HRLCMS of Senna Auriculata were analysed using Agilent 6200 series liquid chromatography system at SAIF IIT Mumbai. The solvent system compared of 95% water (Solvent A): 5% aceonitrile (Solvent B) with applied gradient, flow rate of 0.2 ml/min with column temperature of 25°C. Hypersil gold 3 micron of size 100 x 2.1 MM was used. Injection volume was set to 5μL with run time of 30 mins where the sample ionization was achieved through ESI interface in both positive and negative mode (Kadam et al., 2018; Agme-Ghodke et al., 2016).

**α-amylase inhibitory assay**

According to the previous method (Zhang et al., 2010; Suthindhiran et al., 2009). The extract was mixed with di-methyl sulfoxide solution and different concentrations of the samples were used. The alpha amylase was dissolved in phosphate buffer and the pH was adjusted to 6.8. After pre-incubation 1% of starch solution was added to all the tubes. The reaction mixture was incubated for 15 minutes. Finally, after the incubation time the reaction was stopped using 1 ml of di-nitro salicylic acid reagent and the tubes were kept for boiling in water bath for 10 minutes. The contents were cooled and 10 ml of distilled water was added to all the tubes. Absorbance was measured at 540 nm using microplate reader. Acarbose serves as positive control.

\[ \text{Percentage of inhibition} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \right) \times 100 \]

**α-glucosidase inhibitory assay**

α-glucosidase inhibitory assay was determined using protocol (Li et al., 2005; Suthindhiran et al., 2009). 112μL of potassium phosphate buffer of pH 6.8, 20 μL of enzyme solution (1 unit / ml), 8μL of the aqueous extract of SA were mixed and incubated in 96 well plate at 37°C for 15 minutes. Further 20 μL of p-NPG was added to each well and incubated for 15 minutes at 37°C. The reaction was terminated using 80μL of Na₂CO₃ solution. Absorbance was determined at 405 nm. Instead of sample, 8μL of DMSO was added to control and blank. Acarbose is used as positive control.
**Preparation of Senna auriculata fortified - bread sample**

The bread was prepared using ingredients which is shown in Tables 1, 2 and 3. The ingredients were mixed thoroughly until smooth, which was followed by the rest period of 15 minutes. The dough was then moulded into cylindrical shape into a container of 0.2 mm thick. Then the mould was baked at 120°C for 45 minutes. After baking, the bread loaves were cooled and crust and crumb were separated (Ezhilarasi et al., 2013). The Senna auriculata was added to the wheat flour as levels 2%, 4% and 6% to form Senna auriculata fortified bread sample for investigation studies.

**Table 1: The recipe of 2% Senna auriculata fortified - bread**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>300 grams</td>
</tr>
<tr>
<td>Water</td>
<td>200 ml</td>
</tr>
<tr>
<td>Yeast</td>
<td>7 grams</td>
</tr>
<tr>
<td>Salt</td>
<td>1 %</td>
</tr>
<tr>
<td>Jaggery powder</td>
<td>6.25 %</td>
</tr>
<tr>
<td>Senna auriculata extract</td>
<td>2 %</td>
</tr>
</tbody>
</table>

**Table 2: The recipe of 4% Senna auriculata fortified - bread**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>300 grams</td>
</tr>
<tr>
<td>Water</td>
<td>200 ml</td>
</tr>
<tr>
<td>Yeast</td>
<td>7 grams</td>
</tr>
<tr>
<td>Salt</td>
<td>1 %</td>
</tr>
<tr>
<td>Jaggery powder</td>
<td>6.25 %</td>
</tr>
<tr>
<td>Senna auriculata extract</td>
<td>4 %</td>
</tr>
</tbody>
</table>

**Table 3: The recipe of 6% Senna auriculata fortified - bread**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat flour</td>
<td>300 grams</td>
</tr>
<tr>
<td>Water</td>
<td>200 ml</td>
</tr>
<tr>
<td>Yeast</td>
<td>7 grams</td>
</tr>
<tr>
<td>Salt</td>
<td>1 %</td>
</tr>
<tr>
<td>Jaggery powder</td>
<td>6.25 %</td>
</tr>
<tr>
<td>Senna auriculata extract</td>
<td>6 %</td>
</tr>
</tbody>
</table>
Table 4: Sensory evaluation of Normal white bread with *Senna auriculata* fortified bread

<table>
<thead>
<tr>
<th>Bread samples</th>
<th>Colour(7)</th>
<th>Symmetry(7)</th>
<th>Colour(7)</th>
<th>Texture(7)</th>
<th>Eating quality(7)</th>
<th>Overall acceptability(7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal white bread (control)</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><em>Senna auriculata</em> fortified bread (2%)</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>5</td>
<td>4.5</td>
<td>4</td>
</tr>
<tr>
<td>(4%)</td>
<td>6</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>4</td>
<td>4.5</td>
</tr>
<tr>
<td>(6%)</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>4.5</td>
<td>5.8</td>
</tr>
</tbody>
</table>

The parenthesis values indicates the maximum rating in the score card

Table 5: Bioactive components of White bread and *Senna auriculata* fortified bread

<table>
<thead>
<tr>
<th>Bread samples</th>
<th>Sugars (g/100g)</th>
<th>Reducing sugars (g/100g)</th>
<th>Phenolic content mgGAE/100g</th>
<th>Flavonoids mgCE/100g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal white bread (Control)</td>
<td>4.89±0.08</td>
<td>2.36±0.09</td>
<td>64.21±1.32</td>
<td>20.26±0.56</td>
</tr>
<tr>
<td><em>Senna auriculata</em> fortified bread (2%)</td>
<td>5.32±0.08</td>
<td>2.16±0.11</td>
<td>66.86±1.22</td>
<td>22.81±0.68</td>
</tr>
<tr>
<td>4%</td>
<td>5.46±0.19</td>
<td>2.32±0.13</td>
<td>72.12±1.19</td>
<td>25.64±1.22</td>
</tr>
<tr>
<td>6%</td>
<td>5.89±0.16</td>
<td>2.47±0.10</td>
<td>80.08±1.04</td>
<td>30.32±1.33</td>
</tr>
</tbody>
</table>

Table 6: Starch digestability and predicted glycemic index of *Senna auriculata* fortified bread

<table>
<thead>
<tr>
<th>Bread samples</th>
<th>Total starch %</th>
<th>Resistant starch %</th>
<th>Digestable starch %</th>
<th>Hydrolysis index %</th>
<th>Predicted glycemic index %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal white bread (Control)</td>
<td>65.12±1.02</td>
<td>2.99±1.22</td>
<td>63.12±0.44</td>
<td>44.00±1.09</td>
<td>62.02±1.43</td>
</tr>
<tr>
<td><em>Senna auriculata</em> fortified bread (2%)</td>
<td>70.61±1.32</td>
<td>4.76±1.38</td>
<td>60.68±0.51</td>
<td>42.33±1.19</td>
<td>58.11±0.98</td>
</tr>
<tr>
<td>4%</td>
<td>74.23±1.56</td>
<td>5.04±1.76</td>
<td>68.51±0.62</td>
<td>30.29±2.14</td>
<td>45.18±1.64</td>
</tr>
<tr>
<td>6%</td>
<td>79.18±2.32</td>
<td>10.55±1.20</td>
<td>64.58±0.44</td>
<td>20.16±1.58</td>
<td>50.13±1.10</td>
</tr>
</tbody>
</table>

Weighed and mixed with 8 ml of methanol homogeneously using vortex in 15 ml tube. Followed which the tubes were incubated at 60°C for 5 mins in a water bath. The tubes were then kept in orbital shaker at 300 rpm for 10 mins. The liquid extract and solid fraction were obtained using centrifugation at 3000 rpm for 5 mins using Thermo Fisher Scientific, cooling centrifuge. The liquid extract is used for GCMS analysis.

**GC-MS investigation**

The samples obtained from the centrifugation was analysed using JEOL GCMATE II GC, with maximum resolution of 6000, and maximum calibrated mass 1500 daltons. It is used to analyse complex organic and biochemical mixtures. The compounds from the spectra were collected as they exit the column, which are then identified and quantified based on their mass - to - charge ratio (m/z) (Neelamegam and Ezhilan, 2012).

**Invitro digestability study**

**Invitro digestion of bread**

Based on the modifications in Goh *et al.*, 2015 ; Sui *et al.*, 2016 which was described by Minekus *et al.*, 2015.
Table 7: Evaluation of proximity composition of bread

<table>
<thead>
<tr>
<th>Bread sample</th>
<th>Moisture content %</th>
<th>Crude protein %</th>
<th>Crude fiber %</th>
<th>Ash content %</th>
<th>Lipid content %</th>
<th>Carbohydrate%</th>
<th>Crude fat %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal white bread (Control)</td>
<td>15.10±0.01</td>
<td>12.12±0.01</td>
<td>1.45±0.01</td>
<td>3.44±0.68</td>
<td>2.77±0.55</td>
<td>82.24±0.01</td>
<td>2.38±0.12</td>
</tr>
<tr>
<td>Senna auriculata fortified bread</td>
<td>16.84±0.01</td>
<td>10.40±0.68</td>
<td>1.77±0.02</td>
<td>1.84±0.01</td>
<td>3.11±0.08</td>
<td>85.01±0.58</td>
<td>1.70±0.01</td>
</tr>
</tbody>
</table>

All the sample tubes of the intestinal phase were prepared for 7 digestion time points such 0, 20, 40, 60, 80, 100, 120 mins. All the samples was the centrifuged at 4°C for 10 mins. 4 ml of ethanol was added to the 1 ml supernatant to terminate the reaction. Then they are used for glucose analysis.

Reducing sugar released
The resultant mixture obtained from the intestinal phase was transferred to the dialysis tube and dialysed for 5 hrs at 37°C using PBS buffer. 0.5 ml of dialysate was withdrawn at every 10 mins. The reduced sugar was identified using the method described by Miller, 1959. 0.5 ml of DNS reagent is added to dialysed samples and boiled for 10 mins. The absorbance was determined using Spectrophotometer at 540 nm. Reduced sugar was expressed and calculated in terms of GE, mg/ml.

Sensory evaluation of bread
A score card was used for sensory evaluation for the desirability and undesirability of bread. They are evaluated based on colour, texture, taste, along with quality characeristics like colour of crust, crumb, mouthfeel and overall quality. This was given to panelist consisting for both the genders of age 25 - 50 years. The score for each parameter was assigned by panelist which is evaluated against the maximum score using 7 point hedonic rating scale: excellent as 7, very good as 6, good as 5, satisfactory as 4, fair as 3, poor as 2, very poor as 1 (Rathi et al., 2004; Makinde and Akinoso, 2014).

Estimation of total sugars
The total sugars was estimated based on the protocol (Albalasmeh et al., 2013). 10 μL of sample was combined with 300 μL of 5% phenol. The tube is incubated for 5 mins, further the incubation 1.8 ml of concentrated sulphuric acid was added. The tubes were cooled and absorbance read at 490 nm. Total sugar content was expressed as mg glucose/g
Bioactive components

Estimation of total phenolic content

Modified method of Henriquez et al., 2010 was used. The reaction mixture containing 100 μL of sample, 1 ml of folin - ciocalteau reagent, 2 ml of 10% sodium carbonate solution was incubated for 60 mins at room temperature. Absorbance was measured at 765 nm using UV-Vis spectrophotometer. The values obtained are compared with standard gallic acid. The total phenolic content is expressed as mg gallic acid equivalent/g of extract.

Estimation of flavonoids content

Based on the protocol (Lallianrawna et al., 2013). 75 μL of 5% of NaNO₂ solution was added to 0.9 ml of sample. 150 μL of 10% AlCl₃.6H₂O was added to the mixture after 5 mins, and incubated at room temperature for 5 mins. Followed by 0.5 ml of 1 M NaOH and total volume was made to 2.5 ml using water. The solution was mixed and at 510 nm the absorbance was measured using UV-Vis spectrophotometer. In blank the extracts were replaced by distilled water. The total flavonoids content is expressed as catechin equivalent/g of extract.

Total starch (TS)

Total starch content is determined by the method using protocol (Goñi et al., 1997). 50 mg of ground sample was mixed in 6 ml of 2 M KOH and kept in shaker at room temperature for 30 mins. 3 ml of 0.4 M sodium acetate buffer (pH 4.7) and 60 μL amyloglucosidase (sigma-aldrich) was added. The mixture was incubated at 60°C for 45 mins in water bath. Using glucose oxidase - peroxidase kit the glucose was measured.

Glycemic index (GI)

A modified protocol was used (Goñi et al., 1997). 100 mg of ground sample was incubated with 10 ml of HCl-KCl buffer (pH1.5), 200 μL pepsin solution at 40°C for 1 hour with continuous shaking. pH is raised by adding 200 μL of pancreatic alpha amylase solution, further incubated for 45 mins at room temperature. The reaction of enzyme was stopped by adding 70 μL of Na₂CO₃ solution. 25 ml of tris maleate buffer was added to dilute the samples. 5 ml of pancreatic alpha amylase solution and incubated at room temperature with constant shaking. Aliquots of 1 ml were taken from samples at different time intervals and kept for boiling water with constant shaking for 5 mins. This inactivates the enzyme reaction. Samples were refrigerated. The aliquots were then treated using 3 ml of 0.4 M sodium acetate buffer and 60 μL of amyloglucosidase and further incubated at 60°C, 45 min with shaking. The volume was adjusted to 10 ml using distilled water after incubation period and centrifuged. 0.1 ml of aliquot was transferred into glass tube for glucose measurement using glucose oxidase - peroxidase kit. Absorbance was measured at 510 nm against reagent using UV-Vis spectrophotometer. The values were plotted and concentration over time curve was analysed using sigmaplot 10.0. The samples GI were estimated by the equation of glucose released is compared to the standard glucose that is released (0-180 mins) (Barine and Yorte, 2016).

Quality evaluation of bread

Moisture content

Moisture content of the bread was analysed using the procedure as described by AOAC, 2002.

Crude protein

Protein was determined using micro-Kjeldahl protocol method in AOAC, 2002. The digested protein in the sample was determined spectrophotometrically and calculated as % crude protein = 

\[
\frac{(\text{titre of sample} - \text{blank}) \times 0.01 \times 14.007 \times 6.25}{(\text{10 x weight of sample})} \times 100
\]

Crude fat

This content analysis was carried out using standard method as described in AOAC, 2002.

Crude fiber and ash content

The crude fat and ash content in bread is analysed using the standard method of AOAC, 2002.

Carbohydrate

Using AOAC, 2002 protocol difference method of analysis the bread samples estimation was determined. the crude fiber, protein and fat were subtracted from organic matter; the remaining is calculated as carbohydrate content.

% carbohydrate = 100 - protein % + fat % + ash %

Statistical analysis

The experiments were conducted in triplicates. Using SPSS 20.0 the one way analysis of variance (ANOVA) with Duncan test, which showed p < 0.05 to evaluate the significance of the sample.

RESULTS AND DISCUSSION

Evaluation of invitro - antioxidant activity

The DPPH radical scavenging activity results are shown in Figure 1 and compared with antioxidant
Gallic acid. Low concentration possessed high radical scavenging activity, 90% of radicals were scavenged at lower concentrations. From the analysis, we can conclude that the scavenging effects of *Senna auriculata* extract on DPPH radicals were excellent.

**Evaluation of anti-hemolytic activity**

Hemolysis shows that Senna Auriculata aqueous extract is non toxic in its nature and can be used for further studies. Different concentrations of the extract were analysed for its toxicity studies against human RBC cells. The results showed that control cell which is treated with Triton X 100 which resulted in 100% lysis. The assay results revealed that the *Senna auriculata* extract does not possess any toxicity, which is shown in Figure 2.

**HRLC-MS**

There are around 100 compounds were identified in the extract of *Senna auriculata* by HRLC-MS. Based on the retention time, molecular formula, database formula, most prevailing compounds are identified and the relative compounds found to be with many medicinal properties and the positive and negative ionisations are also depicted below in Figure 3.

**α - amylase inhibitory assay**

The anti diabetic activity of *Senna auriculata* showed the highest inhibition rate of 77.67% was seen at 100 µg/ml concentration and the lowest inhibition rate of 23.67% was seen at 10 µg/ml concentration as shown in Figure 4. Acarbose was taken as positive control. The result showed that the extract is dose dependent and revealed the increase in inhibitory activity against amylase enzyme.

**α - glucosidase inhibitory assay**

Alpha glucosidase plays a major role in modulating PP hyperglycemia. The aqueous extract of *Senna auriculata* was studied for the alpha - glucosidase inhibitory activities which was detected by using pNPG as substrate. The present data revealed that 10, 25, 50 µg/ml showed < 50 % but 100 and 500 µg /ml showed > 50 % of its inhibitory activity as shown in Figure 5.

**Quantification**

**GCMS**

The GC/MS analysis both organic and biochemical mixtures. The name and retention time was shown in Figure 6, exhibits the phytochemical constituents of Senna Aurticulata through GC/MS analysis.

**Invitro digestibility study**

**Invitro digestion of bread**

Figure 7 shows a dose dependent inhibition of *Senna auriculata* during the release of reduced sugar during intestinal digestion of bread. Gastric chyme transit which happens through whole small intestine takes about 4 - 5 hrs (DeSesso and Jacobson, 2001). At 60 mins of dialysate, the concentration of resistant starch of bread sample with *Senna auriculata* exhibited to be low when compared with the control. At the end of 250 mins of dialysis, when compared to control the concentration of resistant starch for Senna Auriculata fortified bread sample 2%, 4% and 6% was reduced.

**Sensory evaluation**

Sensory evaluation of bread samples was given in Table 4. The results were analysed using 7 point hedonic scale. The *Senna auriculata* fortified bread sample is compared with the control which the regular bread based on their crust, colour, symmetry, texture, eating quality, and overall quality of the bread. The global smell was lower in breads prepared using *Senna auriculata* when compared to normal breads. There is a decrease in the flavour of *Senna auriculata* fortified bread when compared with the control due to the taste of the flower, which could be more significant to the sensory attributes and overall acceptability of bread. The formulated fortified bread which is preared using above said concentration was found to have positive effect on consumers overall acceptability, even more among diabetes patients.

**Bioactive components**

Based on the biofunctional properties developed the evaluation is analysed at the retention time of the process. Since they are recommended for the diabetic patients the total sugar and reducing sugar are analysed carefully. The increase in total and reducing sugars depends on the content of *Senna auriculata* which is added on bread. (Table 5) Normal white bread shows a high range of sugar content (6.26 mg/100g), when compared with *Senna auriculata* fortified bread which exhibits low range of sugar content. Reducing sugar also exhibited the same pattern of decrease. Thus the addition of *Senna auriculata* to bread reduces the sugar content. Total phenolic and flavonoids in bread sample of *Senna auriculata* showed a prominent levels of both phenolic content and flavonoids. Many people showed interest in plants for the potential health benefits. Based on those research bioactive components like phenolic, flavonoids etc are more effective and acts as inhibitors of diabetes (alpha glucosidase) and more related health problems (Henriquez et al., 2010).

**Total starch and glycemic index**

Based on the analysis made through sensory
attributes 6% *Senna auriculata* was used for the starch digestibility studies (in vitro). The levels of total starch, resistant starch, digestible starch of the formed bread products are exhibited in Table 6. Total starch and resistant starch ranged about 65.12±1.02 and 2.99±1.22. When compared with the control *Senna auriculata* fortified bread showed higher values in the resistant starch content which resulted in lower digestability starch. The bread prepared showed to be similar in its starch content with the normal or regular breads that are produced. But the digestability of the starch is varied. This kind of differences is due to the protein and dietary fiber content etc. According to the report said by Davis *et al.*, 1994 wheat starch swells slowly when compared to other starches. This is because the protein around the starch granules will stop the granule swelling and gelatinization of starch, further it reduces the enzymatic attack. Based on all these findings the bread produced by the *Senna auriculata* is rich in protein, dietary fiber content. Hence they show raised levels of resistant starch with increase in the concentration of the extract.

Hydrolysis index (HI) is calculated in terms of rate of hydrolysis over the time (Figure 8) along with the predicted glycemic index (pGI) Table 4. Normal white breads exhibited higher hydrolysis index and pGI which exhibited as 44.00±1.09 and 62.02±1.43. *Senna auriculata* fortified bread samples showed lower hydrolysis index and pGI of range 42.33±1.17 - 20.16±1.58 and 58.11±0.98 - 50.13±1.10. Jenkins *et al.*, 2008 reported that food are classified into low (<55), medium (56-69) and high >70. The results shows low pGI which exhibits high resistant starch. Further they even confirm that these biofunctional components act effectively and inhibits the enzyme in post prandial hypoglycemia (Shen *et al.*, 2012). Therapeutic value of low GI in diet of diabetic both type 1 and type 2 are efficient. The low GI as well as high resistant starch reduces the insulin resistance by lower the blood glucose levels in diabetic patients as well as improves their lipid metabolism and to prevent cardiovascular diseases.

**Quality evaluation of bread**

The moisture content of bread shows the life of bread for consumption. The moisture content of bread made with *Senna auriculata* is found to be more when compared to normal bread. To keep the quality of bread moisture content is more important. These moisture content has adverse effect on the storage. The protein content of the wheat flour is 12.12±0.01 and the *Senna auriculata* fortified bread seems to have 10.40±0.68 (Table 7). This results shows that the mixed flour has reduce protein content when compared to the normal bread sample. Even the mixture reduced the fat (1%) content in the flour. There is a decrease in ash content and increase in fiber content when analysed. High ash and crude contents make digestion difficult in human body. There is increase in carbohydrate content due to the extract which makes the bread soft in its texture.

**CONCLUSIONS**

This study indicates that incorporation of *Senna auriculata* into bread reduces the starch digestion and also lowers the glycemic index. It also proves that Senna auriculata has a potential to bakery products with lots of health supplements. The bioactive components of bread like phenolic, flavonoids are found to be higher in the fortified bread sample. Involvement of carbohydrate hydrolysing enzyme which is involved in post prandial hypoglycemia a important cause of type 2 diabetes. This overall invitro findings gives us a promising result that due to the lower glycemic effect the *Senna auriculata* fortified bread sample is situable and recommended for diabetes patients.

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**Conflict of Interest**

None of the authors have any conflict of interest in this manuscript.

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