Enhancing the Nutritional Quality of Carbohydrate Rich Breakfast by Incorporating Mushroom As A Bioactive Functional Additive and Comparing its Glycemic Index

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ABSTRACT
Diabetes mellitus is a metabolic disorder that is increasing globally and is the second leading cause of death worldwide. However, it may be controlled by maintaining glycemic index levels in the daily diet. Idli is one such diet, and is an integral part of the South Indian breakfast. Since it is rich in carbohydrates, cholesterol-free, mildly seasoned, soft on the palette, and easily digestible, it is the best-recommended breakfast meal for hospitalized patients. Mushrooms, on the other hand, are one of the most popular and valuable foods known globally. Mushrooms have low carbohydrate and high protein content, making them an excellent source for diabetic patients. The present study focuses on enhancing the nutritive value of the traditional idli by incorporating three commonly used varieties of mushrooms (white button mushroom, oyster mushrooms, and shiitake mushroom). The glycemic index of plain idli, mushroom idlis, and mushroom varieties was determined in-vitro. Our results suggest that the mushrooms are particularly good for preventing and managing diabetes, especially when incorporated into the traditional idli; they can decrease the glycemic index of idli and thereby help in meal planning.

Keywords: Diabetes Mellitus, Plain Idli, Mushroom Idlis, White Button Mushroom, Oyster Mushroom, Shiitake Mushroom, Glycemic Index.

INTRODUCTION
Diabetes mellitus (DM), which is also known as diabetes, is a metabolic disorder that occurs when the body is not able to take up glucose into its cells and use it for energy. This results in the accumulation of extra sugar in the bloodstream over a long period of time. Low blood sugar, known as hypoglycemia, occurs when the blood glucose drops below a healthy range. If the blood glucose drops too low, it can be a dangerous condition that requires immediate treatment. Hyperglycemia means high levels of blood glucose. Over time, it can cause major health complications in people with diabetes. Several factors can contribute to hyperglycemia, including dietary choices and a sedentary lifestyle.

The signs and symptoms of diabetes include increased thirst, frequent urination, extreme hunger, unexplained weight loss, presence of ketones in the urine, fatigue, irritability, blurred vision, slow-healing sores, and frequent infections, such as gums or skin infections and vaginal infections. Uncontrolled diabetes can lead to serious consequences, causing damage to a wide range of the body’s organs and tissues, including the heart, kidneys, eyes, and nerves.

Regularly monitoring blood glucose levels, recognizing risk factors, selecting appropriate regimens, and balancing glycemic levels might help individuals avoid the serious health complications of diabetes in the future, such as nerve, kidney, and heart damage.

Diabetes can be prevented by maintaining proper glycemic index levels in an individual’s diet. The glycemic index is a system that assigns a number to food containing carbohydrates according to how much each food increases blood sugar or blood glucose level. The glycemic index (GI) of the test food sample can be determined by the hydrolysis index (HI) of a carbohydrate test food.

Carbohydrate-rich breakfast
Idli is a type of savory rice cake, originating from the Indian subcontinent, popular as a carbohydrate-rich breakfast food in Southern India and Sri Lanka. Idli is considered the best food for patients because it is a semi-solid food, typically soft, low in fiber, slightly alkaline, and mildly seasoned. These factors help in preventing increased acid production or reflux and other irritations to the digestive tract. For diabetic patients, consuming idlis 3 to 4 times a day can be unhealthy as it can alleviate their blood sugar levels due to increased carbohydrate intake. However, by lowering the glycemic index and by increasing the dietary fiber as well as the nutritional content of idli, it can be made a healthier option for patients with diabetes. One of the ways by which this can be accomplished is by incorporating mushrooms in powdery form.
Edible Mushroom

Edible mushrooms are the fleshy and edible fruit bodies of several species of macrofungi (fungi that bear fruiting structures that are large enough to be seen with the naked eye). They can appear either below the ground (hypogeous) or above the ground (epigeous), where they may be picked by hand. Edible mushrooms are consumed for their nutritional and culinary value.

Mushrooms are identified all around the world, but only some specific types of them are safe to eat. Mushroom is an essential ingredient in a variety of dishes in multiple cuisines. These are low in carbohydrates, have some protein, and have practically very low fat. They are rich in antioxidants and excellent low-calorie alternatives to meat and other high-fat, high-calorie foods. They are loaded with health-boosting vitamins and minerals which are recognized as an important part of diet. Edible mushrooms are considered a novel source of dietary fiber which help control blood sugar levels and assist in weight management. They contain the highest level of non-starch polysaccharides which are major part of dietary fiber. The remarkable properties of dietary non-starch polysaccharides are water dispensability, viscosity effect, bulk, and fermentability into short-chain fatty acids. In addition, the low glycemic index and high mannitol content of mushrooms are also believed to be beneficial for diabetes.

MATERIALS AND METHODS

Sample preparation

Three different varieties of mushroom powders; White Button Mushroom, Oyster Mushroom, and Shiitake Mushroom were used for the experiment. Machine-dried shiitake mushrooms were obtained from Urban Platter, China; machine-dried oyster mushrooms from Neela D Products, Pune; and white button mushrooms from the local market which were used after being sun-dried for three days. All these mushrooms were finely ground to a powder using a blender. Further, the idli batter was prepared using rice and black gram (2:1 proportion). It was soaked in water for 6 hours and ground to a paste. 1 g each of white button mushroom powder, oyster mushroom powder, and shiitake mushroom powder was added to 3 different bowls containing 50 g of batter. The batter was then allowed to ferment for 9 hours, and the idlis were prepared by steaming for 20 minutes.

Estimation of Moisture Content

An empty, clean, and dry china dish was weighed accurately. A known amount of sample (finely ground) was added to the china dish and weighed accurately. The sample was then dried at 110°C in the hot air oven. This was repeated till constant weight was obtained. From the weights, the moisture content was calculated as %.

Moisture content = \( \frac{(w_2 - w_3)}{w_2} \times 100 \)

Estimation of Ash Content

Dry and clean crucibles were weighed accurately. Approximately 2 g of the sample was taken in a crucible and weighed accurately. The crucibles were dried in the hot air oven at 600°C for an hour till charred. The sample was then cooled and weighed accurately. From the weights, the ash content was calculated as %.

Ash content = \( \frac{(w_2 - w_3)}{w_2} \times 100 \)

Estimation of Dietary Fiber Content

In a crucible, 10 g of the sample was dissolved in 50 ml of 1.25% sulphuric acid. This solution was kept on a hot plate at 70°C for 15 mins, filtered, and to the sediment, 50 ml of 1% sodium hydroxide was added. This solution was again kept on a hot plate at 70°C for 15 mins. Total fiber content was estimated as grams.

Percentage crude fiber = \( \frac{(w_1 - w_2)}{w_3} \times 100 \)

Estimation of Antioxidant Activity

DPPH radical scavenging action Method (Brace. et al., 2002) was used to determine the DPPH scavenging ability of Plain Idli, Mushroom Idlis, and Mushroom Powders. 0.2 ml of the sample extract was taken and was made up to 1.0 ml with distilled water. Aliquots of 0.2-1.0 ml were pipetted out and then made up to 1.0 ml with distilled water. About 3 ml of DPPH was added to all the test tubes, and incubated at room temperature for 10 minutes. The contents of each tube were mixed well before taking the absorbance. The absorbance was then measured at 517 nm against a suitable control and a blank, and the scavenging activity was expressed in %. The absorbance was measured at 517 nm. Ascorbic acid was used as a reference standard for plotting the calibration curve. The percentage inhibition of DPPH by the samples was calculated as follows.

Scavenging effect (%) =\( \frac{[(OD_{of\ control} - OD_{of\ sample}) \times 100]}{OD_{of\ control}} \)

Estimation of Protein

The total protein content was determined using a modified Lowry’s method. 0.5 g of the sample was weighed, ground in a mortar and pestle with 5 ml of 0.01M sodium phosphate buffer (pH 7), and centrifuged at 8000 rpm for 15 mins. 0.2 ml of supernatant was made up to 1 ml with distilled water and mixed with 5 ml of alkaline copper reagent. It was allowed to stand for 10 minutes at room temperature, followed by the addition of 0.6 ml Folin-Ciocalteu reagent (1:1 dilution), and was incubated for 30 mins in the dark at room temperature. The absorbance was then measured at 660 nm. Bovine serum albumin (BSA) was used as a reference standard for plotting calibration curves. The total protein content was determined from the linear equation of a standard curve prepared with BSA.
Estimation of Total Carbohydrates
0.2 ml of the sample solution was used for estimation. The volume in each of the tubes was made up to 1.0 ml with distilled water. A blank test tube was prepared with 1 ml of distilled water. 4 ml of anthrone reagent was added to each of the tubes, following which the tubes were kept in a boiling water bath for 10 mins. The contents of each tube were mixed well, and the absorbance was read at 630 nm against a blank. The readings were noted, and a standard curve was plotted to determine the total carbohydrate content of the samples.

Estimation of Total Phenols
0.2 ml of the sample extract was taken and made up to 1.0 ml with distilled water. To this, 0.5 ml of the FC reagent was added and incubated for 3-5 mins. 2 ml of Na₂CO₃ was added and incubated in a boiling water bath for 1 min, till the color developed. The absorbance was then measured at 650 nm against a blank, and the total phenolic content was expressed in mg/g. Catechol was used as a reference standard for plotting the calibration curve.

Assessment of Glycemic Index
The in-vitro method for evaluating starch digestion is based on proteolysis, followed by incubation with pancreatic alpha-amylase. This method allows the calculation of the Hydrolysis Index (HI), which is indicative of the food’s glycemic index. 2 g of the sample was ground in a mortar and pestle with 20 ml of a 0.1 M potassium phosphate buffer solution (pH 6.9) kept at 37°C was added. After grinding, the samples were homogenized with a homogenizer at a constant speed and rinsed with an additional 20 ml of buffer solution. The pH of the samples was decreased to pH 2.5 with ortho-phosphoric acid, after which 1 ml of pepsin enzyme (Sigma-Aldrich) was added. The samples were placed in a 37°C stirring water bath for 1 hour to stimulate the time that food would take to be churned in the human stomach. Each sample was then buffered back to pH 6.8 with KOH, and 2 ml of the alpha-amylase enzyme (Sigma-Aldrich) was added. The entire contents of the flask were then transferred into a dialysis tube. The tube was closed and placed in a beaker containing 500 ml of buffer solution, which was incubated at 37°C for three hours. Then the samples were extracted every 30 mins. Aliquots of the dialysates were analyzed for reducing sugar by 3’ 5’ dinitrosalicylic acid (DNS) method. The values were plotted on a graph, and the area under the concentration-over-time curve (AUC) was determined.

Sensory Evaluation
Sensory evaluation was carried out for Plain idli, White Button Idli, Oyster Idli, and Shiitake Idli. 17 individuals evaluated the appearance, taste, odor, texture, and overall acceptability, and their observations were recorded carefully. For the evaluation, a 5-Point Hedonic Scale was used.

Table 1: 5-Point Hedonic Scale

<table>
<thead>
<tr>
<th>Score</th>
<th>Hedonic Scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Like Extremely</td>
</tr>
<tr>
<td>4</td>
<td>Like Very Much</td>
</tr>
<tr>
<td>3</td>
<td>Like Moderately</td>
</tr>
<tr>
<td>2</td>
<td>Like Slightly</td>
</tr>
<tr>
<td>1</td>
<td>Neither Like nor Dislike</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION
Estimation of Moisture Content

Figure 1: Comparison of moisture content between the plain idli, white button idli, oyster idli, shiitake idli, white button mushroom powder, oyster mushroom powder, and shiitake mushroom powder.

Figure 1 illustrates the changes in the moisture content of plain idli, mushroom idlis, and mushroom powders which was estimated according to the AOAC, 1984. The highest moisture content among the idlis was found in shiitake idli which is 63.61%, and the lowest value was found in white button idli which is 62.16%. The estimation of moisture content is crucial because, when a person's blood glucose level is high and the kidneys are unable to handle it, water eliminates a huge amount of sugar and ketones from the body. As a result, the moisture content is critical for type 1 diabetes, as it is essential for eliminating excess ketones from the bloodstream and preventing dehydration when blood sugar levels are high. Hence, mushroom idlis are more beneficial for people with diabetes.

Estimation Ash Content
Figure 2 illustrates the variation in the ash content of plain idli, mushroom idlis, and mushroom powders. Among the idlis, the highest ash content was found in plain idli which is 85.26%, and the lowest was found in shiitake idli which is 82.34%. Therefore, incorporating the mushroom powders did not influence the ash content of the traditional idlis.
Figure 2: Comparison of plain idli, white button idli, oyster idli, shiitake idli, white button mushroom powder, oyster mushroom powder, and shiitake mushroom powder.

Estimation of Dietary Fiber

Figure 3: Comparison of the fiber content of plain idli, white button idli, oyster idli, shiitake idli, white button mushroom powder, oyster mushroom powder, shiitake mushroom powder.

Figure 3 illustrates the variation in the dietary fiber of plain idli, mushroom idlis, and mushroom powders. Among the idlis, the highest fiber content was observed in the shiitake idli which is 5.69 % and the lowest value was observed in the case of plain idli which is 3.77%. The incorporation of the mushroom powders in the idlis in general has been shown to increase the crude fiber content of the plain idli. Fiber helps to lower blood cholesterol level, improve blood glucose regulation, and also helps the digestive tract to function properly when eaten in large amounts.

Estimation of Antioxidant Activity

Figure 4 illustrates the changes in the free radical scavenging activity. On determining the values in all the samples, the highest scavenging activity among the idlis was observed in the shiitake idli that is 35.55%, and the lowest in the oyster idli that is 27.77%, suggesting that the radical scavenging activity in these idlis was mainly due to the presence of mushrooms incorporated into them.

Figure 4: Comparison of scavenging activity of plain idli, white button idli, oyster idli, shiitake idli, white button mushroom powder, oyster mushroom powder, and shiitake mushroom powder.

Estimation of Protein

Figure 5: Comparison of the protein content of plain idli, white button idli, oyster idli, shiitake idli, white button mushroom powder, oyster mushroom, powder, and shiitake mushroom powder.

Figure 5 illustrates the variation in protein content. The protein content of plain idli, mushroom idlis, and mushroom powders was determined using Lowry’s Method. Among the idlis, the highest amount of protein content was found in oyster idli which is 245 µg, and the lowest amount of protein content was found in plain idli which is 140 µg. A balanced diet needs to have an adequate amount of protein content since proteins are the major building blocks of the human body and are required for maintaining proper function and immunity in the body. Therefore, idlis essentially serve as a better alternative to traditional idlis because they have a higher protein content.
Estimation of Total Carbohydrate

Figure 6: Comparison of carbohydrate content of plain idli, white button idli, oyster idli, shiitake idli, white button mushroom powder, oyster mushroom powder, and shiitake mushroom powder.

Figure 6 illustrates the variations in the carbohydrate content among samples. When the carbohydrate content of all the samples was calculated, among the idlis, the plain idli had the highest carbohydrate content, which is 10.65 mg, and the oyster idli had the lowest carbohydrate value, which is 9.6 mg. Carbohydrate is a critical component in controlling blood glucose levels since it is turned into glucose and can help manage blood sugar levels. As a result, diabetic diets tend to focus on either the quantity of carbohydrate consumed or the pace at which carbohydrate is absorbed by the body.

Estimation of Total Phenols

Figure 7: Comparison of total phenolic content of plain idli, white button idli, oyster idli, shiitake idli, white button mushroom powder, oyster mushroom powder, and shiitake mushroom powder.

Figure 7 illustrates the differences in total phenolic content in the samples. Among the idlis, the highest amount of phenolic content was observed in shiitake idli which is 5.265 mg, and the lowest amount of phenolic content was observed in plain idli, which is 4.45 mg. The incorporation of mushroom powders into idlis, in general, has been shown to increase the amount of phenolic content in plain idlis. Phenols help to improve human health because of the presence of their antioxidant properties in addition to anti-inflammatory activities.

Assessment of Glycemic Index

Table 2: Comparison of glycemic index between plain idli, white button idli, oyster idli, shiitake idli

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Plain idli</th>
<th>White button idli</th>
<th>Oyster idli</th>
<th>Shiitake idli</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0.286</td>
<td>0.262</td>
<td>0.215</td>
</tr>
<tr>
<td>30</td>
<td>0.333</td>
<td>0.262</td>
<td>0.309</td>
<td>0.333</td>
</tr>
<tr>
<td>60</td>
<td>0.333</td>
<td>0.309</td>
<td>0.379</td>
<td>0.379</td>
</tr>
<tr>
<td>120</td>
<td>0.403</td>
<td>0.333</td>
<td>0.379</td>
<td>0.309</td>
</tr>
<tr>
<td>180</td>
<td>0.45</td>
<td>0.333</td>
<td>0.309</td>
<td></td>
</tr>
</tbody>
</table>

Figure 8: Glycemic response of plain idli, white button idli, oyster idli, shiitake idli

Table 2 and Figure 8 illustrates the glycemic response of plain idli, white button idli, oyster idli, and shiitake idli. The glycemic index value was observed to be significantly lower in white button idli, oyster idli, and shiitake idli when compared with that of plain idli. The incorporation of mushroom powders into plain idli has been shown to reduce the glycemic index value of the plain idli due to the presence of bioactive metabolites in the mushrooms. Therefore, diabetic patients can plan their meals accordingly by combining foods with a higher glycemic index such as idli with those with a lower glycemic index such as mushrooms so as to balance their blood glucose level and prevent hyperglycemia.

Sensory Evaluation

Most members acknowledged the taste and aroma of the white button mushroom idlis, and out of the 17 candidates, 10 candidates extremely liked the taste. 10 candidates extremely liked the aroma of the white button idli, which was found to have the highest score. The score for overall acceptability was found to be excellent for white button mushroom idli in comparison to other mushroom idlis. The next preferred idli was shiitake idli followed by oyster idli in terms of taste, aroma, and overall acceptability.
Sensory evaluation is essential as it helps to understand the palatability of a particular food, which makes it easier for the researcher, especially in the research and development sector to understand the demand for a particular food in a population.

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