Production and Assessment of Nutritional, Antidiabetic and Antioxidant Properties of Biscuit Fortified with some Antidiabetic Plants for Diabetic Patients

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The study aimed to produce biscuit that fortified with three different concentrations of mixed antidiabetic medicinal plants including cinnamon, sage, moringa leaves, fenugreek, avarampoo, bitter gourd, panakilangu and ghritkumari for diabetic and evaluate the nutritional, antioxidant and antidiabetic characteristics of the biscuit. After preparation and baking of biscuit samples, a proximate composition, physical attributes, antioxidant and antidiabetic capacity such as phenol, flavonoid, ABTS scavenging capability, FRAP, α-amylase inhibition, α-glucosidase inhibition and glycemic index were measured. Our results demonstrated that the moisture, fat, carbohydrate content were found to significantly ($p<0.05$) decline as concentration of medicinal plant powder increased while the protein, fiber and ash content were found to significant ($p<0.05$) increase with increase the concentration of medicinal plant powder. According to the findings, the use of medicinal plants in high concentrations resulted in noticeably higher levels of phenol and flavonoid in addition to DPPH, ABTS radical scavenging activities and FRAP values. α-amylase and α-glucosidase enzymes are highly inhibited by the medicinal plant biscuit. Fortifying biscuits with medicinal plants may play an important role in controlling sugar levels where a high level of α-amylase enzyme inhibition and antioxidants may result in a lower blood glucose level. This could be a trend for diabetes control in the future. Therefore, it is anticipated that the antidiabetic, antioxidant-rich biscuits made in this study will offer consumers many important health advantages.

1. Introduction

Diabetes is one of the most common chronic diseases in the world, affecting approximately 285 million people, and the number is growing. The main factor that lead to diabetes regulation is the quantity and quality of food, especially carbohydrates and fats. Many peoples use medicinal plants in their traditional treatments because they are inexpensive compared to pharmaceuticals, contain a lot of antioxidants, antidiabetic, and anti-inflammatory compounds, and have fewer side effects than chemicals (Gull et al., 2015). In recent years, there has been a distinct trend in the food industry to produce functional foods such as fortifying biscuits in this study with medicinal plants, which contain many bioactive compounds that have a positive effect on human health. Ghritkumari (Aloe vera) is one of several medicinal plants with anti-diabetic properties that can lower blood glucose levels (Rashidi et al., 2013). Moringa (Moringa oleifera) leaves have a good ability to control high blood glucose levels because they contain good amounts of antioxidants like ascorbic acid and chlorogenic acid, as well as polyphenols like rutin and kaempferol (Adelaja & Schilling, 1999). Cinnamomum verum, or cinnamon, has been shown in some studies to be a natural insulin stimulant (Broadhurst, Polansky, & Anderson, 2000). It has been demonstrated that the water-soluble components of cinnamon increase the effectiveness of the insulin signaling pathway (Imparl-Radosevich et al., 1998). Cinnamon contains procyanidin type-A polymers, which are thought to increase insulin receptor autophosphorylation and, consequently, improve insulin sensitivity. Among the herbal remedies is fenugreek (Trigonella foenum-graecum). It contains compounds like galactomannan and saponins (Rashid et al., 2019) that give it a variety of qualities, including anti-diabetic and antioxidant qualities (Kumar et al., 2021). Another study highlights that sage (Salvia officinalis L.) possesses diverse biological properties, such as hypoglycemic, anti-inflammatory, and antioxidant effects (WHO, 2002). Becuase it contains lectin, which can reduce appetite, bitter gourd (Momordica charantia) is regarded as one of the medicinal plants with antidiabetic properties (Ooi, Yassin, & Hamid, 2012).

A popular food item, biscuits have a number of appealing qualities, such as high nutritional value, palatability, variety in flavors and shapes, affordability, and extended shelf life (Akubor, 2003). Furthermore, consumers have been demanding more nutrient-dense and functional foods in recent years. Taking into account biscuits' appealing qualities as well as the wide range of consumer ages, biscuits can be used as school snacks for underweight schoolchildren, or as an adult and elder snack (Baljeet, Ritika, & Roshan, 2010). Therefore, a variety of bioactive substances and medicinal plants can be added to the biscuit product to fortify it. Consequently, the objective of this research was to create biscuits that fortified with medicinal plants and evaluate the nutritional, antioxidant and antidiabetic characteristics of the biscuit.

2. Materials and Methods

2.1. Preparation of Medicinal Plant Leaves Powder

Medicinal plants : Cinnamon (*Cinnamomum verum*), sage (*Salvia officinalis*), moringa (*Moringa oleifera*) leaves, Fenugreek (*Trigonella foenum-graecum*), Avarampoo *(Cassia auriculata*), bitter gourd (*Momordica charantia*), Panakilangu *(Borassus flabellifer*) and ghritkumari (*Aloe vera*) were collected from different locations of Baghdad according to WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants, Geneva, 2003. After being cleaned, the powdered leaves of medicinal plants were dried for 12 hours at 60°C. Before being used, each plant's dried powder was ground, sifted, and kept it at 4 C.

Table 1: Suggested Combinations for Biscuits.

Components of biscuits	Control	Sample A 1%	Sample B 3%	Sample C 5%
Flour (g)	300	297	291	285
Cinnamon (*Cinnamomum verum)* (g)	-	0.375	1.125	1.875
Sage (Salvia officinalis L.) (g)	-	0.375	1.125	1.875
Moringa Leaves (*Moringa oleifera*) (g)	-	0.375	1.125	1.875
Fenugreek (*Trigonella foenum-graecum*) (g)	-	0.375	1.125	1.875
Avarampoo *(Cassia auriculata)* (g)	-	0.375	1.125	1.875
Bitter gourd (*Momordica charantia*) (g)	-	0.375	1.125	1.875
Panakilangu (*Borassus flabellifer*) (g)	-	0.375	1.125	1.875
Ghritkumari (*Aloe vera*) (g)	-	0.375	1.125	1.875
Milk (ml)	15	15	15	15
Butter (g)	9.375	9.375	9.375	9.375
Egg	3	3	3	3
Salt (g)	2.25	2.25	2.25	2.25
Baking powder (g)	3	3	3	3
Water (ml)	60	60	60	60

2.2. Manufacturing of Biscuits

As indicated in table 1, the process described by Chauhan, Saxena, & Singh (2016) was used to produce biscuit combinations using varying concentrations of the medicinal plant mixture. After combining the ingredients, let the dough ferment for three hours. After that, it was cut and molded to the proper dimensions. The samples were allowed to cool to room temperature and placed in appropriate bags for storage after the biscuits were baked for 15 minutes at 150–200°C.

2.3. Measurement of Proximate Composition of Biscuits

According to Nwosu, Edo, & Özgör (2022), biscuits were chemically examined to measure the moisture, fat, protein, and ash. The AOAC method (AOAC, 2016) was used to determine fiber, and the difference was used to measure carbohydrates.

2.4. Measurement of the Biscuits' Physical Attributes

A precise balance was used to weigh the biscuit samples, and the average height of six biscuits was used to estimate thickness. Using a ruler, the biscuits' diameter was measured in accordance with Ayo et al. (2007) and the spread ratio value was determined by dividing the diameter of biscuit by the thickness (Mildner-Szkudlarz et al., 2013).

2.5. Measurement of Total Phenol

Using the method outlined by Singleton, Orthofer, & Lamuela-Raventós (1999) to measure the total phenol in biscuit samples, suitable dilutions of the biscuit samples' aqueous were oxidized using 2.5 ml of 10% Folinciocalteau, neutralized, and then incubated for 40 minutes at 45°C. The total phenol was then calculated by determine the amount of absorption at 765 nm.

2.6. Measurement of Total Flavonoid

Using the method reported by Meda et al. (2005) to measure total flavonoids, 0.5 ml of the biscuit sample solution was added to a solution that contained 1.4 ml of distilled water, 50 µl of 1 mol/l CH_3COOK, 50 µl of 10% $AlCl_3$, and 0.5 ml of methanol. After 30 minutes, absorbance of the mixture was determined at 415 nm.

2.7. Measurement of DPPH Free Radical Scavenging Capacity

The technique referenced by Aluko & Monu (2003) was employed to assess the capacity to scavenge free radicals, by adding 1 ml of the biscuit sample solution to 1 ml of the 0.4 mM methanolic solution included DPPH radicals. After 30 minutes, absorbance was determined at 516 nm.

2.8. Measurement of ABTS Scavenging Capability

The procedure described by Re et al. (1999) involved mixing 2.45 mM of $K_2S_2O_8$ with 7 mM of ABTS solution and leaving it in the dark for 16 hours. the absorbance at 734 nm was recorded after combining 0.2 milliliters of sample solution with the ABTS blend.

2.9. Measurement of Ferric Reducing Antioxidant Power (FRAP)

The technique described by Zhang, Wang, & Xu (2008) was applied, in which 2.5 milliliters of the biscuit sample solution were mixed with 2.5 milliliters of 1% potassium ferricyanide and 200 milliliters of sodium phosphate. For 20 minutes, the solution was kept at 50 °C. 2.5 ml of 10% $C_2HCl_3O_2$ was added to the resultant solution. Following centrifugation, the absorbance at 700 nm was measured after adding 5 ml of distilled water and 1 ml of 0.1% ferric chloride.

2.10. Determination of α-amylase Inhibition

Cisneros-Yupanqui et al. (2023) described a procedure in which 100 µL of biscuit sample solution was blended with a solution made of combining 100 µL ml of Na_3PO_4 with amylase solution (1 mg/mL) and 0.006 M sodium chloride. Ten minutes were spent with the solution at 25 °C. Additionally, 0.006 M sodium chloride, 20 mmol/L Na_3PO_4, and 100µL of 1% starch solution were combined, and the blend was then allowed to sit at 25°C for 10 minutes. 200 µL of ($C_7H_4N_2O_7$) was then added to the solutions. For five minutes, bring the mixture to a boil. Add three milliliters of distilled water once it has cooled. At 700 nm, absorbance was recorded.

The subsequent equation was utilized to measure the inhibition value.

Alpha glucosidase activity inhibition % = Abs (control1) - Abs (sample) x 100/ Abs (control).

2.11. Measurement of α-glucosidase Inhibition

50 µl of each sample and enzyme were transferred to 96 wells using the technique reported by Cisneros-Yupanqui et al. (2023). The blend was then kept at 37 °C for 10 minutes, mixed with nitrophenyl-glucopyranoside, and then incubated once more at 37 °C for 30 minutes. Before and after incubation, the absorbance at 405 nm was recorded. In the control, buffer is used rather than enzyme. The subsequent formula was utilized to determine the inhibition value:

Alpha glucosidase activity inhibition % = Abs (control1) - Abs (sample) x 100/ Abs (control)

2.12. Measurement of Glycemic Index (GI)

The technique described by Bakar, Ahmad, & Jailani (2020) was applied, in which 1 g of pepsin in 10 ml of Hydrochloric Acid-Potassium Chloride Buffer was mixed with sample and then kept at 40 C for 60 min and after that the volume was completed to 25 ml with phosphate buffer, 5 ml of α- amylase was added. The solution was kept at 37 ◦C for 0-3 h, The solution was combined with 30 ml of amyloglucosidase and sodium acetate buffer. After heating the sample to boiling for 5 min and measuring glucose level, the blend was centrifuged. At 540 nm, the supernatant's absorbance was recorded. The subsequent formula was utilized to record the starch hydrolysis and GI values

Hydrolysis Index (HI) (%) = AUC (sample) x 100/ AUC (ref)

GI = 39.71 + 0.549 HI

Where: AUC = Area under curve

2.13. Statistical Analysis

Three duplicates of each experiment were conducted. One-way analysis of variance (ANOVA) was performed using SPSS version 19, and a p-value of less than 0.05 was deemed significant. The mean± SD was used to provide the data.

3. Results and Discussion

Table 2 shows the composition of the medicinal plant biscuits samples. Our findings showed that as the amount of medicinal plant powder elevated, the moisture, fat, and carbohydrate decreased. The moisture was ranged from 5.61 in sample C to 8.20% in control. According to Alam et al. (2014) fortified biscuits with herbal are less moist than regular biscuits. Because of its low moisture content, it may possess an extended shelf life and demonstrate greater resistance to microbial spoilage (Alpers et al., 2021).

Table 2: Composition of Plant Medicinal Biscuit.

Composition (%)	Control	Sample A	Sample B	Sample C
Carbohydrate	64.28±0.10[a]	63.30±0.17[b]	62.51±0.23[c]	62.86±0.13[c]
Fat	14.85±0.04[c]	14.61±0.08[c]	13.23±0.20[b]	12.63±0.14[a]
Moisture	8.20±0.16[a]	7.53±0.28[b]	6.37±0.17[c]	5.61±0.12[d]
Protein	10.47±0.13[d]	12.23±0.08[c]	13.79±0.15[b]	14.52±0.08[a]
Fiber	1.59±0.21[a]	2.21±0.08[b]	2.44± 0.11[b]	3.18± 0.18[c]
Ash	0.91±0.04[d]	1.69±0.09[c]	2.46±0.15[b]	3.20±0.07[a]
Energy (kcal)	429.95±0.18[d]	421.46±0.23[c]	416.07±0.15[b]	410.19±0.21[a]

The variations in the moisture-holding capacity of various ingredients could be the cause of the variations in moisture amount among samples (Navarathna & Rathnayake, 2017). Our findings showed that the moisture content of the control and treatments made with medicinal plants powder differed significantly ($p<0.02$). These results align with those of Owheruo et al. (2023). The range of fat content was 14.55 to 11.63. The low fat content of medicinal plants may be the cause of the degreasing fat content in biscuits. Excessive fat content can cause oxidative rancidity and the formation of disagreeable and pungent compounds in the product (Ikuomola, Otutu, & Oluniran, 2017).

According to our findings, the fat content of the control and medicinal plant powder treatments differed significantly ($p<0.02$). These findings are consistent with those of (Ahmed et al., 2022). The range of the carbohydrate content was 64.24 in the control and 61.86 in sample C. The low carbohydrate content of medicinal plants may be the cause of the degreasing carbohydrate content in biscuits. The amount of carbohydrates in each biscuit sample varied significantly. Ganorkar & Jain (2014) demonstrate the protective health effects of food products with high fiber and low carbohydrate content. These findings concur with those of Alam et al. (2014) and Owheruo et al. (2023), who discovered a declining trend in the carbohydrate level of biscuit samples created from plant and mushroom parts. However, our findings showed that adding medicinal plant raised the fortified biscuits' ash, protein, and fiber amount. The biscuit samples had protein contents ranging from 10.47 to 14.52. The protein amount of all biscuit samples increases significantly ($p<0.05$) when medicinal plants are added. Given that the medicinal plant contains a sizable amount of protein, this increase was anticipated. This finding suggests that adding medicinal plants to biscuits would enhance their nutritional value. These findings are in line with those of Ahmed et al. (2022) and Karki et al. (2016), who noted an upward trend in the protein amount of biscuit samples with plants.

Samples of biscuits had dietary fiber contents ranging from 2.50 to 4.95%. The findings showed that the dietary fiber content of the control and medicinal plant biscuit samples differed significantly ($p<0.05$). The trend of rising dietary fiber content suggests that using medicinal plants had a major impact by raising the dietary fiber

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of various percentages of all biscuit samples. This could be because medicinal plants contain a lot of fiber. These findings concur with those of Owheruo et al. (2023) and Agu et al. (2020), who discovered an upward trend in the fiber amount of biscuit samples derived from plant parts. According to Li & Komarek (2017) dietary fiber serves a significant function in the human body by lowering blood cholesterol and blood glucose levels, preventing obesity, and lowering the risk of colon cancer

As the amount of medicinal plants in the biscuit samples increased, so did their ash content. At every level of substitution, the ash content of the samples and the control varied significantly ($p<0.05$). The biscuits' ash content varied from 0.91 to 3.20 percent. These findings are in line with those of Alam et al. (2014) who noted an upward trend in the amount of ash in biscuit samples made from plant parts. The food samples' mineral content is indicated by their ash content (Mishra, Devi, & Jha, 2015). Minerals are vital nutrients that support the body's vital metabolic processes. According to the findings, consumers will receive more minerals from biscuits made with medicinal plants than from regular biscuits.

Table 3: Physical Characteristics of Biscuits.

Variables	Control	Sample A	Sample B	Sample C
Thickness (cm)	0.63±0.03^{a}	0.60±0.02^{a}	0.0.51±0.03^{b}	0.42±0.02^{b}
Diameter (cm)	5.09±0.04^{a}	5.23±0.04^{b}	5.37±0.02^{c}	5.28±0.05^{d}
Spread ratio (g/cm)	6.39±0.03^{d}	6.73±0.04^{c}	6.97±0.06^{b}	6.75±0.03^{c}
Weight (g)	7.36±0.04^{a}	8.28±0.03^{b}	9.19±0.04^{c}	9.23±0.02^{c}

The physical attributes of medicinal plant biscuits are displayed in Table 3, where the weight, diameter, and spread ratio all increased while the thickness decreased. Increasing the biscuits' diameter and spread ratio is thought to be a reliable way to gauge their quality and arise the biscuit. The incorporation of medicinal plants may have increased the biscuit's protein, which is why its weight increased.

Table 4: Antioxidant Ability of Biscuit.

Variables	Control	Sample A	Sample B	Sample C
TPC (mg GAE/100gm)	37.56±0.08^{d}	40.78±0.04^{c}	44.47±0.06^{b}	49.17±0.7^{a}
TFC (mg QE/100gm)	23.46±0.05^{d}	28.14±0.09^{c}	32.94±0.04^{b}	35.89±0.10^{a}
DPPH (%)	36.69±0.09^{d}	38.76±0.05^{c}	41.23±0.03^{b}	44.58±0.02^{a}
ABTS(AEE/g)	0.38±0.02^{d}	0.47±0.01^{c}	0.61±0.03^{b}	0.72±0.05^{a}
FRAP(AEE/g)	29.98±0.32^{d}	33.47±0.14^{c}	39.69±0.21^{b}	45.84±0.24^{a}

3.1. Antioxidant Characteristics of Medicinal Plant Biscuit

Table 4 shows the antioxidant characteristics of the medicinal plant biscuit. In comparison to the control sample, the total phenol of the biscuit samples rose as the concentration of the medicinal plant powder increased. The biscuits' total phenol content varied between 37.56 and 49.17 mg GAE/100 mg, with significant differences ($p<0.05$) between samples A, B, and C and the control. Similarly, as table 4 illustrates, when the concentration of medicinal plants increased, the biscuit samples' total flavonoid content increased as well, ranging from 23.46 to 35.89 mg QE/100 mg, with significant differences ($p<0.05$) between the samples and the control. Table 4 displays the DPPH radical scavenging capabilities of biscuit aqueous extracts. The DPPH radical scavenging activity of the medicinal plant-fortified biscuits was higher than that of the control, ranging from 36.69 to 44.58%. There was a significant difference ($P < 0.05$) in the DPPH values between the control and biscuit samples. According to our findings, there is a strong correlation (Adefegha & Oboh, 2013) between the biscuit samples' total phenol and DPPH radical scavenging activity; samples with the highest phenol content and the highest concentration of medicinal plant powder also had the highest DPPH radical scavenging activity, while samples with the lowest phenol content showed the lowest DPPH radical scavenging activity. Table 4 displays the results of medicinal plant biscuits' ABTS radical scavenging activities, which varied from 0.38 to 0.72 AEE/g and demonstrated a significant difference ($P < 0.05$) between samples and between control and biscuit samples. Table 4 displays the FRAP of medicinal plant biscuit results, which varied from 29.98 to 45.84 and increased as the medicinal plant powder concentration rose in comparison to the control sample. Additionally, our findings indicated that the FRAP values of the control and biscuit samples differed significantly ($P < 0.05$).

Table 5: Carbohydrate Inhibitory Enzyme of Biscuit.

Variables	Control	Sample A	Sample B	Sample C
α-Amylase inhibition (%)	39.57±0.18^{d}	44.12±0.26^{c}	48.94±0.14^{b}	54.68±0.10^{a}
α-Glucosidase inhibition (%)	26.78±0.11^{d}	31.43±0.07^{c}	34.91±0.13^{b}	39.36±0.14^{a}

3.2. Carbohydrate Inhibitory Enzyme Activities of Medicinal Plant Biscuit

Table 5 displays the medicinal plant biscuit's capacity to inhibit carbohydrate enzymes. Alpha amylase and alpha glucosidase enzyme inhibitory are highly concentrated in the medicinal plant biscuit; the alpha amylase inhibitory value varied from 39.57 to 54.68%, while the alpha glycosidase inhibitory value varied from 26.78 to 39.36%. After eating, the high percentage of alpha amylase inhibition might aid in reducing the absorption of carbohydrates. Alpha amylase and alpha glucosidase inhibitors are frequently used to stop complex carbohydrates from breaking down into quickly absorbed monosaccharides (Gong et al., 2020). Consequently, post-meal blood glucose levels may be lowered by slower glucose absorption (Edo et al., 2022). Alpha amylase and alpha glucosidase inhibitors' primary effects are to lower postprandial blood glucose levels (Inoue et al., 2022). This implies that these enzymes' actions work in concert to reduce blood glucose levels. According to our findings, medicinal plant biscuits exhibit a strong inhibitory effect on the two primary digestive enzymes; as a result, they may be a promising trend for diabetes management in the future (Lund & Ray, 2017).

Table 6: Glycemic Index and Glycemic Load of Biscuits.

Variables	Control	Sample A	Sample B	Sample C	Glucose
Glycemic index %	53.15±0.23^{d}	42.95±0.17^{c}	38.79±0.11^{b}	35.49±0.09^{a}	100
Glycemic load %	30.07±0.14^{d}	25.16±0.12^{c}	23.94±0.05^{b}	21.08±0.08^{a}	50

The glycemic load (GL) assesses carbohydrates in relation to the glycemic index and the quantity of food carbohydrates, whereas the glycemic index (GI) gauges how quickly blood sugar (glucose) can rise. Table 6 displays the biscuits' glycemic index and glycemic load. According to the study's findings, biscuits' glycemic indexes (GI) and glycemic load (GL) dropped as the amount of medicinal plant substitution rose. The glycemic index ranged from 35.49% in sample C to 53.15 % in control. According to their GI classification, foods are categorized as low GI (55), medium GI (56–69%), and high GI (>70%). Since all of the samples' GI values were less than 55, according to our results, we can classify them as low glycemic index samples, making them appropriate and acceptable for diabetic patients. Glycemic load (GL) varied from 21.08% in sample C to 30.07% in control.

4. Conclusion

The study's findings demonstrated that adding a variety of medicinal plants to biscuits enhanced their nutritional value, texture, antioxidant capacity, and antidiabetic effects. We can draw the conclusion that the overall antioxidant activities of biscuits were enhanced by an increase in the concentration of medicinal plants. As a result, it is anticipated that the antioxidant-rich biscuits made in this study will offer consumers general health advantages. Slower glucose absorption may lower post-meal blood glucose levels, which may be a potential future direction for diabetes control. The results also showed that adding medicinal plants to biscuits improved their antidiabetic properties by lowering the glycemic index and glycemic load and by increasing the level of carbohydrate inhibitory enzyme activities like α-amylase and α-glucosidase, which are accountable for the disintegration and absorption of carbohydrates.

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