International Journal of Chemical and Biochemical Sciences
(ISSN 2226-9614)
Journal Home page: www.iscientific.org/Journal.html

Evaluation of anti-diabetic potential of *Syzygium cumini* seed (SCS) powder infused tortilla as therapeutic diet to mitigate hyperglycemia

Hmna Saleem[1], Nuzhat Jamil[2], Ayesha Ahmad[3], Hafiz Muhammad Saad Ali[4], Irsa Zafar[5], Mudasir Majeed[6], Muhammad Yasir[4]*, Muhammad Hamza[7], Qudsia Kanwal[4]

[1]*Department of Nutritional Science, Government College University, Faisalabad, Pakistan*

[2]*Nanotechnology Division, National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan*

[3]*Department of Chemistry, Superior University, 17Km Raiwind Road, Kot Araian, Lahore, Pakistan*

[4]*Department of Chemistry, The University of Lahore, Lahore, Pakistan*

[5]*Department of Chemistry, Government College University, Faisalabad, Pakistan*

[6]*Department of Applied Chemistry, Government College University, Faisalabad, Pakistan*

[7]*Additive Manufacturing Institute, Shenzhen University, Shenzhen, China*

Abstract

Diabetes mellitus, a chronic metabolic disorder, is characterized by hyperglycemia. Current therapies to cope up the disease are inadequate to provide instant glycemic control resulting in severe health damage. In current study, *Syzygium cumini* seed (SCS) powder supplemented wheat tortillas were evaluated qualitatively and quantitatively to regulate the blood glucose levels in diabetic patients. For this purpose, wheat flour was blended with SCS powder at different treatment levels (2%, 4% and 6%). All treatments (T_1 – T_3) were subjected to proximate analysis, rheological attributes and sensory evaluation to get most acceptable supplementation. Results of such studies revealed that T_1 treatment (2% CSC) was found to be the most suitable one, showing paramount results for rheological attributes (Final viscosity: 1803.02±8.84 mPa.s; Peak viscosity: 1597.3±9.99 mPa.s), proximate analysis (Moisture content: 25.82±1.10 %, Ash content: 2.19±0.08%, Fiber content: 1.22±0.08%) and sensory features (Overall acceptability: 8.0±0.07). Tortillas made by such optimized blending level (2% CSC), when applied to a given set of diabetic patients, exhibited remarkable results by giving a significant decrease (206.17±6.01 mg/dL at day1 while 180.41±5.08 mg/dL at day21) in blood glucose level upon increasing time of treatment. Thus, *Syzygium cumini* seeds can be effectively blended in various food products to control diabetes and other related diseases.

Keywords: Anti-diabetic potential; Proximate analysis; Rheological behavior; Sensory evaluation; Tortilla.

Full length article *Muhammad Yasir, e-mail: muhammad.yasir@chem.uol.edu.pk Doi # https://doi.org/10.62877/25-IJCBS-24-25-19-25

1. Introduction

High blood glucose level is an outcome of chronic obstruction of pancreatic activity in which regulation of insulin is disrupted due to imbalance of starch, protein and lipid metabolism. It is categorized into Type I diabetes (insulin dependent) which was caused when insufficient insulin is produced by abnormal functioning of β cells. Such type of diabetes is mostly inherited type in which body cells attack pancreatic cells to destroy their normal function in auto-immune fashion. Diabetic ailment caused when subjected to exogenous sources like obesity and too much storage of glucose in liver, results in Type II diabetes (non-insulin dependent). Symptoms of Type I appear more quickly, and can be managed by taking exogenous insulin while Type II can be overcome by control in diet, exercise, proper medication and modifying the lifestyle [1-2].

More than 90% diabetic population is suffering from Type II which is more common and relates to the current unhealthy lifestyle, especially in urban regions. Glucose uptake in large quantities in shape of fast food items, without an adequate extent of workout results in typical type of insulin independent diabetes. Problems with normal body hormone, insulin results in deposition of high glucose levels in different body tissues which incorporate veins, renal medulla, visual focal point, cardiovascular tissues and peripheral nerves which result from serious and supported hyperglycemia [3-4]. *Syzygium cumini* is well renowned for its medicinal attributes especially during diabetes since ancient times, thus, had been the important ingredient of many folk medicines of diabetes. The biochemical analysis showed that its major therapeutic constituent, jamboline has radical scavenging behavior. Hence it was known to have hyperglycemic, liver protective, anticancer, antiviral, anti-allergy and anti-inflammatory activity [5-6]. The flavonoids present in *S. cumini* seeds extract were found to exhibit high reducing potential and metal chelating activity. Another study demonstrated a significant reduction of serum glucose level and increase secretion of insulin as a result of dietary intake of flavonoids [7-8]. The increased secretion of insulin was found to relate the reduced destruction of thymus and improves the working of Immune system [9-10]. The role of *Syzygium cumini* seeds was evaluated against diabetes mellitus in experimental rats by using ethanolic extract of *S. cumini* seeds. There was a significant reduction in blood glucose level as *S. cumini* seeds has the potential to decline the activity of certain salivary enzymes *i.e.* glutamate oxaloacetate transaminase (GOT) and glutamate pyruvate transaminase (GPT) [11]. Modern research revealed that *Syzygium cumini* seeds improved hyperinsulinemia as *S. cumini* seeds comprised of multiple flavonoids, alkaloids and glycosides which have potency to control and regulate insulin secretion by pancreatic cells [12-13]. In developing countries like Pakistan, due to unawareness about causes and symptoms of diabetes as well as high cost of treatments, different ways are required to be established, comprising of some diet based therapies so that every person can easily afford it. Following these key points, the current research was planned to address hyperglycemia through feeding trials of *Syzygium cumini* seed (SCS) powder infused tortillas to selected diabetic patients.

2. Materials and methods

2.1. Pretreatment and sample preparation

Syzygium cumini fruits were collected from local trees of university botanical garden, University of Agriculture, Faisalabad, Pakistan and seeds were separated manually, washed with distilled water, dried in hot air oven, and finally ground to get fine powder which was packed in air tight jar and stored at ambient temperature for further studies.

2.2. Preparation of supplemented tortillas

Syzygium cumini seed (SCS) powder was mixed with wheat flour at variable concentrations i.e0%, 2%, 4% and 6%. Such composite flour samples were used to make tortillas of regular sizes. Round tortillas of 20 cm diameter were developed by following the already reported methods of Kapoor et al. [14] and Dominguiz-Hernandez et al. [15]. Briefly, 100g of each of composite wheat flour samples (0%, 2%, 4% and 6 %) was mixed with desired quantity of distilled water to make viscoelastic dough; amount of water taken up by each sample was regarded as % water absorption (Table 1). In order to get efficient dough, physical kneading was performed for 2.5 minutes and resultant dough of each sample was kept at 30ºC for 30 min at 78% atmospheric humidity. Finally, dough of each flour sample, was rounded manually, charted by sheeting/rolling on the leveled surface and baked on open hot girdle. Time of baking and intensity of flame underneath girdle were carefully controlled.

2.3. Viscoelastic behavior of supplemented doughs

Viscosity of all composite sample and control doughs was investigated by carefully observing the viscograms obtained applying rapid visco analyzer (RVA 4500). Briefly, each flour sample (3g) was mixed with 25mL distilled water in aluminum sample bin. Rapid visco analyzer (RVA) stirs the slurry at the temperature of 60-95ºC for 3:42 min, maintained at 95ºC for 2:30 min, then cooled at 50ºC in 3:48 min, and held at 50ºC for 2:00 min. The RVA was operated with paddle speed of 960 rpm for first 10s then 160 rpm for the remaining experiment [16]. The viscosity of control and composite flour doughs was expressed in terms of peak viscosity (maximum viscosity obtained during pasting), breakdown viscosity (difference between peak viscosity and lowest viscosity), final viscosity (viscosity obtained at the end of pasting cycle), setback viscosity (difference between peak viscosity and final viscosity) and pasting temperature (the temperature at which the viscosity of flour sample begins to rise).

2.4. Physicochemical analysis of tortillas

Thus prepared tortillas were analyzed for their physical and chemical attributes by following different investigation tools [17].

2.5. Proximate analysis

The control and supplemented tortilla samples were analyzed for moisture, crude fat, crude protein, crude fiber and ash contents by following the already reported method of [18].

2.6. Color analysis

The color analysis of tortilla samples as carried out by using the Lab scan XE spectro-colorimeter (Hunter Associate Laboratory Virginia, Model: LX16244) following the method described by [19-20]. Color evaluation was carried out by measuring Hunter values (L*, a* and b*). Degree of greenness to redness was indicated by a* values (ranges from -100 to +100) while b* (-100 to +100) values represented the blueness to yellowness coloration; and that of L* (0 to 100) values demonstrated blackish to lightest shades.

2.7. Texture analysis

Texture analysis was performed by suing texture analyzer with needle probe (Stable Microsystems Model: TA-XT2, Surrey, UK). Puncture test was performed for texture analysis using a 2mm needle probe. Strips measuring 2cm x 2cm were cut from each tortilla and placed in sample cell of texture analyzer one after other. The speed of the probe movement reaches upto 2mm/s while it works in normal mode. Texture analysis was interpreted in terms of force required to penetrate the needle into tortilla strips placed in sample cell of texture analyzer [21].

2.8. Sensory evaluation

The purpose of sensory evaluation is to document different attributes of thus formulated finalized product. Such analysis involved the investigation about flavor, color, texture, hardness, sense of taste and overall acceptability of final developed tortillas. For this purpose, a panel of seven skilled food technologists from National Institute of Food Science and Technology (NIFST), University of Agriculture Faisalabad (UAF) was developed. A 9-point hedonic scale was developed according to [22-23], in order to rank all the formulated samples of tortillas; score 9 represents the highly recommended sample while number 6 shows as the lowest score (poorly accepted sample); number 5 shows neutral one while number 1 describes the extremely negative sample.

2.9. Administration of optimized tortilla to diabetic patients

The best treatment T_1 (SCS 2%), optimized on the basis of sensory and proximate analysis, was investigated by administrating the type 2 diabetic patients (n=12) who participated voluntarily in the current research trials and were selected on the basis of recommended inclusion and exclusion criteria. The patients were divided into two groups: one (n=06) for control study while other (n=06) for optimized treatment T_1. Tortillas made by following control (SCS 0%) and treatment T_1 (SCS 2%) were feeded to the patients twice a day for a study period of 21 days in equal quantities. Blood samples (2mL) were collected after day 0, 7, 14 and 21^{st} day and preserved according to standard clinical protocols using commercially available sample vials; and serum from all samples was separated to measure glucose and insulin levels.

2.10. Clinical laboratory analysis

Serum glucose and insulin levels were examined from blood samples collected after day 1, 7, 14 and 21^{st} day at the end of the treatment. Commercially available Bioclin® Glucose mono-reagent diagnostic kit was used to measure the serum glucose level having limit of detection (LOD) within the range of 2-500 mg/dL with % CV < 3.11. While serum insulin was measured through commercially available ELISA kits, E-Lab Sciences catalog # E-EL-R2466 to quantify serum insulin having sensitivity of 0.47µLU/mL, detection range of 0.78-50 µLU/mL and % CV < 10.

2.11. Statistical analysis

All the data collected was analyzed by one-way analysis of variance (ANOVA). While differences among the means of groups by an unpaired, two-sided student's t-test; $p < 0.05$ was considered statistically significant. The statistical analysis was performed to investigate the level of significance of recorded values [24].

3. Results and Discussions

3.1. Rheological behavior of supplemented doughs

The flour viscosities show indirect relation with pasting temperature. On heat treatment, the swelling power and water absorption was observed on each cooking cycle (from 50 to 90ºC).

3.1.1. Peak Viscosity

During pasting of composite flours, the maximum viscosity achieved is regarded as peak viscosity. Continuous heating and pasting results in complete hydration of all starch granules present in flour converting it into thick slurry. Thus, peak viscosity also gives an indication of water holding capacity of composite flour [25-26]. Results (Table 2) have shown that peak viscosity increased with increasing contents of CSC powder into wheat flour (1383.30±552.99 mPa·s for T_1 to 1597.90±648.78 mPa·s for T_3) which can be attributed to the fact that *Syzygium cumini* seed powder has quite low hydration capacity than flour cellulosic contents. Such results of peak viscosity are quite comparable to peak viscosity of pasta blends (1,491–868 mPa.s) as reported by Bawa et al. [27]. It has been found that more efficient dough is only possible with considerable hydration capacity of flour, thus T_1 is more suitable than other two treatments (T_2 and T_3).

3.1.2. Break down Viscosity

The difference between peak viscosity and lowest viscosity reached during settling stage is termed as break down viscosity. The continuous heating under isothermal conditions, after peak viscosity has been achieved, there was a large dip in the viscosity of flour paste, mainly resulted from collapsing of flour granules and leaching of solubilized polymeric molecules from hydrated turgid granules into the solution. The reduction in viscosity relates to alignment of polymeric molecules in the direction of applied shear, and such viscosity decline is termed as breakdown viscosity. The extent of breakdown and swelling depends on the types and blending of flour, temperature and shear force [28-29]. Results indicated that treatment T_1 (2% SCS) showed lowest breakdown viscosity (507.67±12.2 mPa·s) of all treatments which coincides with the findings of Akonor et al. [30] for starch contents extracted from different cassava species (354–520 mPa·s); where minimum breakdown viscosity depicts the least adherence and susceptibility of paste towards heat deterioration. It also indicates the limited destruction of starch granules.

3.1.3. Final Viscosity

The last stage, during viscosity analysis by RVA, involves an isothermal cooling where viscosity becomes constant after sharp continuous increase. This highest value of viscosity is regarded as final viscosity. In this phase, starch granules of flour form a viscous paste that leads to an abrupt increase in viscosity [31-32]. Analysis of variance (ANOVA analysis) of final viscosity of composite flours, depicted a significant increase in viscosity from T_1 (1383.30±552.99 mPa·s) to T_3 (1597.90±648.78 mPa·s) treatment which resembles the results of Gomez et al. [33] for reduction stream (R7.1) of milled rye flour (1509 mPa·s). The lowest value of final viscosity for treatment T_1 facilitates easy puffing of resulting tortillas.

Table 1: Rheological attributes of *Syzygium cumini* added wheat flour dough at different levels of supplementation

Treatment	Water absorption (%)	Peak viscosity (mPa.s)	Breakdown viscosity (mPa.s)	Final viscosity (mPa.s)	Set back viscosity (mPa.s)	Pasting temperature (°C)
T_0	79.45±1.45[cd]	1636.33±13.82[a]	615.05±3.74[a]	2005.33±9.80[a]	883.33±4.8[c]	69.55±1.22[c]
T_1	80.78±1.65[c]	1597.30±9.99[ab]	597.67±11.02[b]	1803.02±8.84[b]	898.15±4.8[c]	70.97±2.16[c]
T_2	82.12±0.95[b]	1389.86±9.58[c]	538.49±12.87[c]	1698.81±11.2[c]	953.88±5.2[b]	78.31±2.59[b]
T_3	83.45±1.24[a]	1383.90±12.78[cd]	507.65±12.2[d]	1629.13±12.6[cd]	986.66±6.2[a]	83.15±2.85[a]

Here T_0 = Wheat flour (100%), T_1 = SCS (2%), T_2 = SCS (4%), T_3 = SCS (6%)

Table 2: Rheological attributes of *Syzygium cumini* added wheat flour dough at different levels of supplementation

Treatment levels	Water absorption (%)	Peak viscosity (mPa.s)	Breakdown viscosity (mPa.s)	Final viscosity (mPa.s)	Set back viscosity (mPa.s)	Pasting temperature (°C)
T_0	79.45±1.45[cd]	1636.33±13.82[a]	615.05±3.74[a]	2005.33±9.80[a]	883.33±4.78[cd]	69.55±1.22[c]
T_1	80.78±1.65[c]	1597.30±9.99[ab]	597.67±11.02[b]	1803.02±8.84[b]	898.15±4.78[c]	70.97±2.16[c]
T_2	82.12±0.95[b]	1389.86±9.58[c]	538.49±12.87[c]	1698.81±11.22[c]	953.88±5.24[b]	78.31±2.59[b]
T_3	83.45±1.24[a]	1383.90±12.78[cd]	507.65±12.2[d]	1629.13±12.62[cd]	986.66±6.21[a]	83.15±2.85[a]

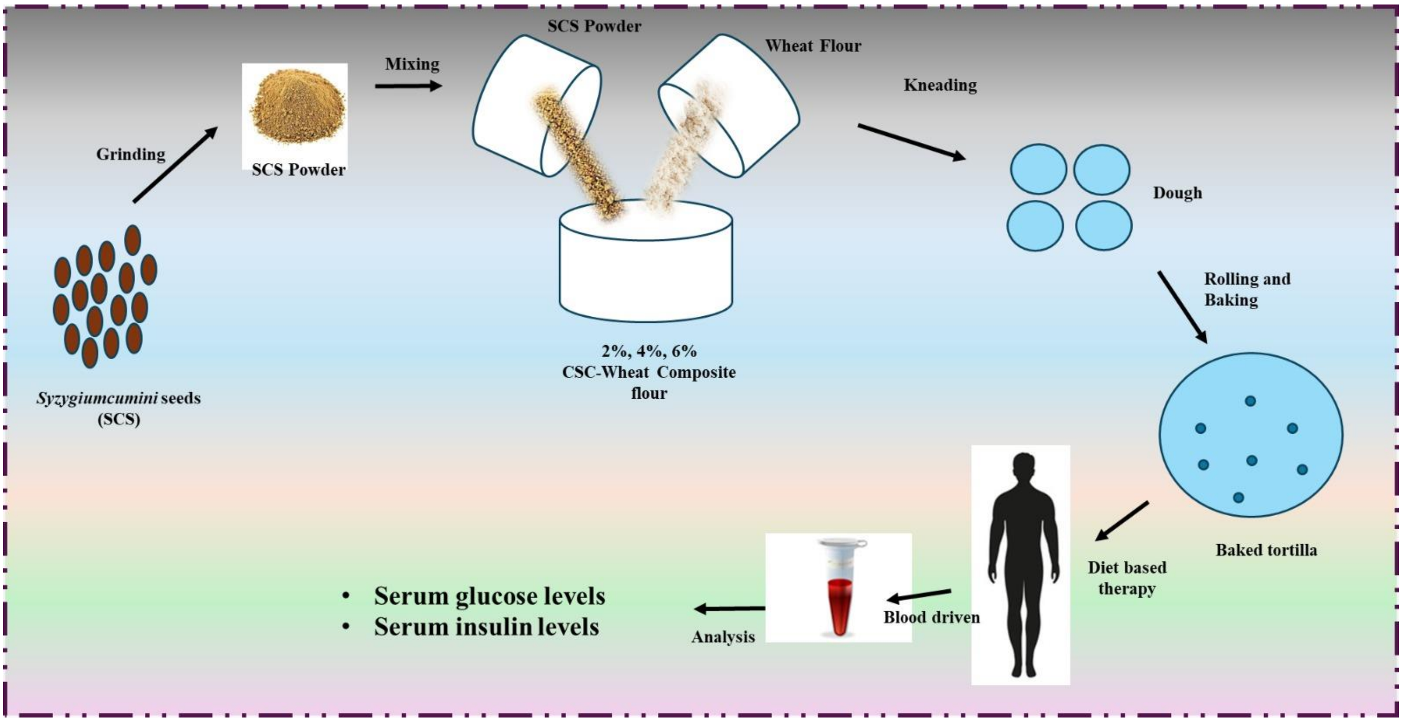
Here T_0 = Wheat flour (100%), T_1 = SCS (2%), T_2 = SCS (4%), T_3 = SCS (6%)

Table 3: Proximate, color and texture analysis of *Syzygium cumini* supplemented wheat tortillas

Treatments	Proximate contents (%)					Color Analysis			Texture analysis (Kg/mm^2)
	Moisture	Ash	Crude Fiber	Crude Protein	Crude Fat	L*	a*	b*	
T_0	24.37±0.49[d]	1.65±0.06[d]	0.77±0.09[d]	8.1±0.16[a]	0.67±0.02[a]	73.8±0.48[a]	2.42±0.04[d]	17.53±0.28[a]	0.31±0.008[d]
T_1	25.82±1.10[bc]	2.19±0.08[c]	1.22±0.08[c]	7.76±0.17[ab]	0.62±0.02[a]	62.52±0.22[b]	3.27±0.11[c]	15.38±0.1[b]	0.37±0.009[c]
T_2	26.19±1.17[b]	2.87±0.09[b]	2.25±0.11[ab]	7.42±0.22[b]	0.61±0.03[a]	55.91±0.26[c]	4.11±0.06[b]	14.38±0.02[c]	0.42±0.02[ab]
T_3	27.10±1.03[a]	3.34±0.11[a]	2.68±0.12[a]	7.14±0.24[bc]	0.6±0.03[a]	52.24±0.25[cd]	5.24±0.25[a]	14.23±0.02[c]	0.43±0.04[a]

Table 4: Impact of selected treatment of CSC supplemented wheat tortillas on serum glucose and insulin levels as a function of application time intervals

Treatments	Time Interval	Glucose Level (mg/dL)	Insulin Level (μLU/mL)
Control	Day 1	206.17±6.01[a]	9.17±0.62[a]
	Day 7	205.48±5.22[a]	9.47±0.54 [a]
	Day 14	202.76±6.24[a]	9.32±0.64 [a]
	Day 21	204.42±5.66[a]	9.87±0.13 [a]
Treatment T_1	Day 1	206.17±6.01[a]	9.88±0.64[a]
	Day 7	197.16±4.19[b]	10.06±0.44[a]
	Day 14	188.14±5.41[c]	10.12±0.32[a]
	Day 21	180.41±5.08[d]	9.24±0.56[a]



Graph 1: Graphical abstract of the study

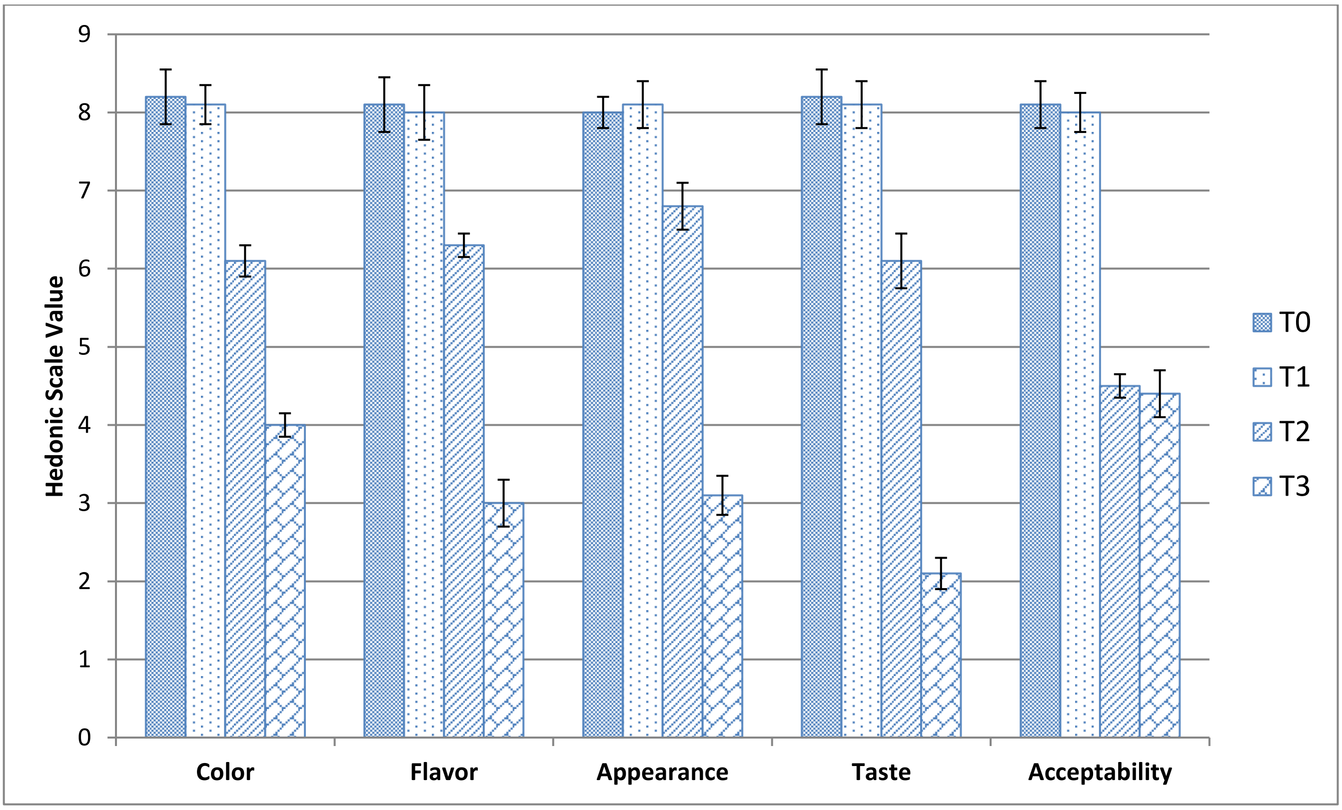


Figure 1: Sensory evaluation of *Syzygium cumini* seed powder supplemented wheat tortillas on the basis of hedonic scale

3.1.4. Setback viscosity

The difference between final viscosity and peak viscosity is termed as setback viscosity. Higher values of setback viscosity indicate the higher tendency of swollen granules to revert back to original shape while lower values indicate the higher retention tendency [34-35]. The linear increase in setback viscosity of supplemented flour from treatment T_1 (983.33±4.78 mPa.s) to treatment T_3 (886.66±368.2 mPa.s) showed the behavior of gluten to maximize the activity of starch retro-gradation with time and higher retention capacity of T_1 composite flour. These values of setback viscosity are in line with previous report of Shar et al. [36] for super rice variety crossed with tropic japonica variety (889±36.76 mPa.s).

3.1.5. Pasting temperature

Pasting temperature in the one, at which viscosity of flour begins to increase. When polymeric granules are completely gelatinized, viscosity begins to rise and such temperature value is regarded as gelatinisation temperature which is mostly lower than that of pasting temperature; hence, pasting occurs just after gelatisation [37-38]. ANOVA results of supplemented tortillas (Table 2) indicated a non-significant decrease in pasting temperatures from T_1 (71.97±28.16 ºC) from T_3 (69.15±25.85 ºC). Similar results for pasting temperature of different categories of potato flours (66.83±0.11ºC for steamed potato flour; 66.78±0.04ºC for freeze dried potato flour and 70.08±0.11 ºC for low gelatinization potato flour) were also presented in the literature report of Zhang et al. [39].

3.2. Physiochemical analysis

3.2.1. Proximate evaluation

Proximate characterization was performed in terms of moisture, ash, crude fiber, crude protein and crude fat contents. Current findings indicated that as supplementation was increased from 0 to 6 % (T_0 to T_3), moisture contents were raised significantly (24.37±0.49% for T_0 to 27.10±1.03% for T_3), that corresponded to the fact that CSC powder has high affinity for water molecules. Similar trend was observed in case of ash content which also increased with increasing concentration of CSC powder i.e. 1.65±0.06% for T_0 to 3.34±0.11% for T_3. Since CSC is rich in fiber content that's why crude fiber content was also increased with increasing concentration of CSC powder (0.77±0.09% for T_0 to 2.68±0.12% for T_3). Proteins and fats are important nutritional ingredients of cereals. Both protein and fat contents deceased non-significantly with increasing supplementation levels. This decrease in protein and fat contents is related to high concentration of fibers in CSC, which is also a health benefit for humans.

3.2.2. Color analysis

The composite tortillas were analyzed at different levels of added SCS powder. The current findings (Table 3) indicated a decreasing trend of L* value (lightness) by increasing the concentration of SCS powder in tortillas (73.8±0.48) to T_3 (52.24±0.25) which accounted for the fact that darkness of tortillas was increased with increasing CSC powder concentration. These observational changes takes place during Millard reaction, which can be explained as the reaction between amino acids and reducing sugars in the process of baking that gives tortillas a distinctive flavor [40]. In contrast to this, a* (greenness to redness) showed an increasing trend *i.e.* from 3.27±0.11 for T_1 to 5.24±0.25 for T_3, when CSC powder concentration was increased from 2% to 6% in the normal wheat flour. The increase in redness is related to increasing carotene contents in T_3 supplemented flour which is in line with increasing concentration of CSC powder. Whereas, b* (blueness to yellowness) exhibited a decreasing trend from T_1 (15.38±0.11) to T_3 (14.23±0.02). Dark blue shade of T_1 composite corresponded to higher anthocyanin contents of this supplemented treatment.

3.2.3. Texture analysis

The texture of supplemented tortillas was analyzed in terms of their hardness by measuring the force of penetration by texture analyzer. As SCS powder has higher fiber content so it resulted in increased hardness of supplemented tortillas with increasing concentration of SCS contents. According to the current results, increasing trend of SCS concentration directly increases the hardness of tortillas i.e. 0.37±0.009 kg/mm^2 for T_1 to 0.43±0.04 kg/mm^2 for T_3 supplementation. T_3 (SCS 6%) tortillas became so harder that hindered their dietary applications.

3.4. Sensory Evaluation

Sensory analysis is of great significance for any type of human food. Sensory analysis parameters provide healthy information and insights about a food product that is crucial for food's overall acceptability. Sensory characterization of CSC supplemented tortillas has been presented in Figure 1, which clearly demonstrated a close resemblance of T_1 supplementation level to whole wheat tortilla. All sensory features including color, flavor, taste, texture, overall appearance and acceptability are in close proximity to normal whole wheat bread which strongly recommends its oral administration to type II diabetic patients. Other two supplemented tortillas T_2 and T_3, although showing good physicochemical properties but they are widely deviated from normal whole wheat tortilla in their sensory characteristics.

3.5. Serum glucose and insulin analysis

Administration of optimized fortified tortillas to selected set of six patients as compared to control (whole wheat) to other set of selected six patients resulted in some alterations in the concentration of both diabetic parameters. Results of clinical analysis (Table 4) indicated serum glucose level of all selected patients was decreased (206.17±6.01 mg/dL at day1 and 180.41±5.08 mg/dL at day21) with increasing administration period of selected treatment as compared to control treatment (206.17±6.01 mg/dL at day1 and 204.42±5.66 mg/dL at day21); while serum insulin level remained un-affected by increasing treatment time. Analysis of variance (Table 4) indicated significant decrease ($p \leq 0.05$) in blood glucose level from day1 (206.17±6.01 mg/dL) to day21 (180.41±5.08 mg/dL) while insulin analysis exhibited non-significant variations from day1 (9.88±0.64µLU/mL) to day21 (9.24±0.56µLU/mL).

4. Conclusions

Supplementation of wheat flour with *Syzygium cumini* seed (CSC) powder induced anti-diabetic properties in such composite flour tortillas. Such bioactivity of thus produced tortillas is highly attributable to phytochemical composition and nutraceutical values of *Syzygium cumini* seeds which are already reported in literature. T_1 treatment (2 % CSC powder) was observed to be the most suitable one in terms of its sensory evaluation and rheological attributes. Administration of such optimized treatment of tortillas resulted in significant decrease in blood glucose level, strongly recommending its applications to control diabetes in human beings. Thus, application of *Syzygium cumini* seeds should not only restricted to tortillas, but can also be applied to other food products, not only to control diabetes, but also to overcome other diabetes originated harmful diseases.

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