Egypt. J. Chem. **Vol. 68**, No. 3 pp. 245 - 262 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Biochemical Assessment of Functional Chocolate Balls Fortified with Microencapsulated Synbiotic in Diabetic Rats

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Abstract

phytochemicals and synbiotics based food products offer excellent choices for treating diabetes. In this study, we aimed to produce a novel baked balls prepared with brown rice and pumpkin flour as phytochemicals sources and covered with dark chocolate fortified with synbiotic microcapsules as a functional food for diabetics.

Nutritional, antioxidant and sensory characteristics for novel functional baked balls were determined. The synbiotic microcapsule was prepared by mixed the probiotic cells pellets encapsulated with sodium alginate and whey protein as well as the prebiotic maltodextrin at 3% (w/v) and the viability of synbiotic microencapsulated during exposure to stimulated gastrointestinal juices (SGJ) was estimated. The biological effects of the functional baked balls were studied using four groups of Sprague-Dawley rats. Group one was employed as the control. The other three groups were injected with 45 mg/kg of streptozotocin. Group 2 was employed as un-treated diabetic while groups 3 and 4 fed on a balanced diet supplemented with 20% of the baked balls covered with dark chocolate with or without symbiotic microcapsules for 6 weeks.

The results revealed that a prepared backed balls as good source of micro and macronutrients, including vitamins, minerals, dietary fiber, lipids, ash, and protein. Furthermore, showed antioxidant power and the content of phenolic compounds was 31.488 mgGAE/g. The encapsulation materials for probiotic cells were able to protect the cells after exposure to SGJ and the survival rates for microcapsules was 63.93%. The prepared balls showed hypoglycemic and anti-inflammatory effects in diabetic rats, which was evident by inhibiting the increase in malondialdehyde, nitric oxide, tumor necrosis factor-alpha, C-reactive protein, interleukin 6 and transforming growth factor β1.

Finally, the novel functional baked balls seem to be promising in diabetes therapeutic via enhance composition of gut microbiota and reduction of inflammation and oxidative stress related to diabetes.

Keywords*: Dietary intervention- Probiotic – Synbiotic – Diabetic - baked chocolate balls – Microencapsulation-Antioxidant activity.*

1. Introduction

An autoimmune condition known as type 1 diabetes (T1D) is defined by the immune system's destruction of pancreatic beta cells. Through the activation of an aggressive adaptive immune response against β-cells, interactions between genes and the environment have a significant impact on immune system dysfunction [1]. T1D is becoming more common everywhere, primarily as a result of the paucity of effective preventive and therapeutic methods. The occurrence of T1D increased during the last 25 years by 3.7% in children (boys and girls) aged 0 to 4, 3.4% among boys and 3.7% among girls aged 5 to 9, and 3.3% among boys and 2.6% among girls aged 10 to 14 as illustrated by the EuroDIAB (European Diabetes) findings [2]. The intestinal mucosal immune system and the bacteria that live there closely interact. Early in life, the gut microbiota colonizes and the immune system develops simultaneously; hence, changes in the gut microbiota can affect the functioning of immune cells and vice versa. In T1D patients, abnormal gut microbiota alterations (dysbiosis) are frequently found, especially in those who have been identified as multiple-autoantibody-positive due to an active and unfavorable immune response. The pathophysiology of T1D involves the activation of self-reactive T-cells, which causes CD8+ T-lymphocytes to destroy β -

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Received date 15 May 2024; revised date 26 June 2024; accepted date 21 July 2024
DOI: 10.21608/EJCHEM.2024.289930.9714

cells. Additionally, it is becoming obvious that gut bacteria and T-cells have a strong relationship [3]. Probiotics support the regulation of microbial metabolic activities, such as the generation of advantageous short chain fatty acids (SCFAs) and the maintenance of a balanced gut microbiota [4]. Prebiotics are indigestible fermented food ingredients including dietary fibers made of non-starch polysaccharides, that specifically encourage the development, make up, and activity of microflora in the gastrointestinal tract, hence enhancing the health of hosts [5]. Probiotics and prebiotics are combined to form synbiotics. They contribute significantly to the metabolization of many dietary substrates and hence to the preservation of human health [6]. A decrease in the autoimmune response and gut integrity have been linked to the treatment of gut dysbiosis with certain probiotics and prebiotics [7].

Plants, particularly those with high amounts of antioxidant components and dietary fiber, play a major role in improving diabetes disorders associated with oxidative stress [8]. Furthermore, that eating dark chocolate is high in flavanols lowers the chance of developing diabetes and cardio-metabolic disorders [9]. In a prior study [10] discovered that baked items are not only cheap to prepare, but they are also long-lasting, and easy to fortify as a useful functional food. Therefore, our work focused on produce the novel functional baked balls covered with fortified chocolate by synbiotic microcapsules and studied effect on gut microbiota and biomarkers of inflammation and oxidative stress in diabetes rat model.

2. Materials and methods

2.1. ***Materials:*** All materials, used to prepare the chocolate balls, were obtained from a local market in Cairo, Egypt. From Agri-mark, USA, we obtained whey protein concentrate (WPC, 80 %). Sodium alginate (SA) was purchased from Loba Chemie, Pvt Ltd - Mumbai, India. Prebiotic maltodextrin agent was purchase from Loba Chemie, Mumbai, India. All the chemicals and reagents used in this study were of fine analytical grade.

Probiotic strains

The mixture of probiotic strains (Lactobacillus acidophilus CH-2, Lactobacillus rhamnosus NRRL B-442, Lactobacillus gasseriB-14168, Bifidobacterium lactis BB12 and Bifidobacterium bifidum NRRL B-41410) was obtained from Dairy Department, National Research Centre.

Animals and Animal's diet

Male albino Sprague-Dawleyrats, weigh 147±10.5g as (Mean ± SD) were used. Rats were provided from the animal house of National Research Centre, Cairo, Egypt. The animals were housed individually in stainless steel cages at room temperature. Water and food were always available.

The balanced diet was formulated and prepared from 10% casein, 10% corn oil, 10% sucrose, 60.5% maize starch, 5% fiber, 3.5% salt mixture(AIN-93) and 1% vitamin mixture(AIN-93) [11].

2.2. Methods:

2.2.1. Preparation of cells pellets formicroencapsulation

The probiotic strains (*Lactobacillus acidophilus, Lactobacillus rhamnosus, Lactobacillus gasseri, Bifidobacterium lactis and Bifidobacterium bifidum*) were activated individually using MRS broth and incubated for 24 h at 37ºC anaerobically to obtain high biomasses. The cell pellets were obtained by centrifugation at 5000 rpm, for 15 min at 4ºC. The obtained cells pellets were washed by a sterile saline solution (0.9% (w/v) NaCl) and stored at 8ºC for encapsulation procedure.

2.2.2. Microencapsulation procedure

Probiotic cells pellets were encapsulated with sodium alginate andwhey protein as coating materials. Whey protein concentrate (WPC) 80 % was dissolved in sterilized water (10 % w/v). The solution was stirred for 1 h at room temperature and rest for 2 h to confirm the protein hydration. WPC solution was heated to 80°C for 45 min to denature the proteins and cooled overnight. Then, Sodium alginate (SA) was added to the denatured WPC solution (3 % w/v) and stirred to complete dissolution [12].

Freshly probiotic cells pellets were mixed well with equal volumes and added to the prepared wall materials solution (sodium alginate with whey protein) with the concentration (25 g / 100 ml) to obtain cells concentration 25 %. Additionally, the prebiotic maltodextrin was added to the prepared mixtures of wall materials and cells pellets with the concentration 3% (w/v). All mixtures were blended well using homogenizer for 2 min at 1500 rpm (Ingenieurbüro CAT, Germany). The obtained microencapsulated synbiotic mixture was frozen at -20 °C /24 h and then freeze-drying using a lyophilizer(ALPHA

1-4 LSC) for 36h. Then, the obtained synbiotic microencapsulated powder was stored at 4 ℃ in sterilized bottles for the next experiments and in vivo study, described in Figure 1.

2.2.3. Encapsulation characterizations

- *Microencapsulation yield (% MY)*

One gram of the resulting synbiotic microencapsulated powder was dissolved in 9 ml of sterile tri sodium citrate solution (2%, w/v) and stirred, which was considered the first dilution (1:10). Followed by normal serial dilution using saline solution (0.9 % NaCl) and added the appropriate dilution to petri dishes to determine the probiotics counts in microencapsulated powder using deManRogosa Sharpe (MRS) agar medium and pour plate method. The petri dishes were incubated at 37°C/ 48h under anaerobic conditions. The % MY for probiotic strains was calculated as the following:

MY(%) = log10N/Log10No ×100

Where: N = the number of the probiotics cells inside the microencapsulated powder, No = the number of the probiotics cells pellets before added to the wall materials.

- *Morphological pattern of synbiotic microencapsulated powder*

The Morphological pattern for resulted microencapsulated powder was determined using scanning electron microscope (SEM, JEM-2100 electron microscope; Jeol, Tokyo, Japan).

- *In vitro study for the viability of synbiotic microencapsulated powder during exposure to stimulated gastrointestinal juices*

The gastric and intestine solution were prepared according to [13]. The synbiotic microencapsulated powder and other free cells used as control were tested their survivability in the prepared gastrointestinal solutions. Firstly, 10 g of synbiotic microcapsules or free cells was added into 100 ml gastric solution for 2h at 37 ºC. After that time, one gram of synbiotic microcapsules and free cells were collected to determine the survival rate using MRS agar and pour plate method. Secondly, the gastric solution was replaced by intestine simulated solution and the synbiotic microcapsules and free cells were incubated at 37 ºC for another 6h. One gram of microencapsulated powder and free cells were collected for each 2h intervals. After collecting the sample of encapsulated and free cells, the survival rate was detected by MRS agar medium using pour plate method. The viable cells count was determined after 48 h of incubation at 37 ℃ and performed in duplicate.

2.2.4. Preparation of baked chocolate balls fortified with microencapsulated symbiotic

The baked balls were prepared from 30g of brown rice flour, 24g peanut butter, 15g fresh mashed pumpkin, 10g eggs, 15g skim milk, 1g baking powder and 2g stevia powder (sachets). The brown rice flour and the baking powder were mixed in a bowl, and then the peanut butter, fresh mashed pumpkin, eggs, skim milk and stevia sugar were added to obtain dough. The dough was cut into equal volumes balls that baked at 150°C for 15 minutes, The prepared baked balls were divided to two portions the first portion was covered with melting dark chocolate (5 gm per one bar) that fortified with (1g, containing more than 106 CFU of probiotics) of powder microencapsulated synbiotic (MSB) and the other portion were covered with melting dark chocolate (5 gm per one bar) without fortification with microencapsulated synbiotic as a control (CB). The baked balls were allowed to cool before packaging in polyethylene bags for 21 days of storage at room temperature.

2.2.5. Proximate analysis of baked chocolate balls

The baked balls covered with chocolate fortified with microencapsulated symbiotic were analyzed to determine their composition (moisture, crude protein, crude fat, ash) contents according to the methods described [14]. All analyses were performed in triplicate and values are expressed as g/100 g dry sample. Total carbohydrate content was calculated by difference method. Total dietary fiber (TDF), insoluble dietary fiber (IDF) and soluble dietary fiber (SDF) for baked chocolate balls were determined according [15].

Macro and trace minerals content (K, Ca, Mg, P, Na, Fe and Zn) of baked chocolate balls was determined using an absorption Spectrometry (Pyeunicm Model 3300, PyeUnicam Ltd. Cambridge, England)) atomic absorption spectrophotometer equipped. The samples were prepared according to [16].

2.2.6. Fat- and water-soluble vitamins content of baked chocolate balls

The study analyzed the fat- and water-soluble vitamins in baked balls coated with chocolate fortified with microencapsulated symbiotic using HPLC. The Agilent C18 column was used for fat-soluble vitamin A and E analysis. The mobile phase was methanol: acetonitrile 65:35. While, water-soluble vitamins (B1, B2, B6 and B12) HPLC analysis was performed using an Agilent 1260 series, with ZORBAX SB-C8 for separation. The mobile phase was water with 0.01% TFA and Methanol, programmed for water-soluble vitamins in a linear gradient.

2.2.7. Antioxidant properties of baked chocolate balls

- *Extraction of phenolic compounds*

The extraction procedure was carried out according to modified method [17]. Chocolate balls sample (2.0 g) were mixed with 20 mL of methanol (80%) for 24 h at room temperature. The procedure was repeated twice. methanol extracts were centrifuged at 4000 g for15 min, and the resulting supernatants were pooled. The supernatants were stored for total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity (AA).

- Total phenolic content (TPC)

TPC of chocolate balls extract was determined spectrophotometrically using Folin-Ciocalteu reagent [18]. Gallic acid was used as a standard and results were expressed in mg of gallic acid equivalents (GAE) per g of sample on dry mass basis.

- Total flavonoid content (TF)

TFC was determined by Aluminium chloride (AlCl3) calorimetric test [19]. The results of the TFC were expressed as rutin equivalents (mg RE/g dry).

- Antioxidant activity (AA)

AA of the phenolic extract, based on the scavenging activity of the stable 1,1-diphenyl-2- picrylhydrazyl (DPPH) free radical [20],the antioxidant activity was calculated according to the following equation:

AA (%) = (Abs control – Abs sample)/ Abs control x 100

2.2.8. Oxidative Stability of lipid fraction of chocolate balls during storage

The peroxide value (PV), thiobarbituric acid (TBA), total phenolic content (TPC), and radical scavenging activity (RSA) were used to measure the stability of the lipid fraction for baked balls covered with chocolate reinforced with microencapsulated throughout the course of 21 days. Hexane was used as an extracting solvent in the Soxhlet method of extracting lipids. The PV was calculated using the titration method in accordance with ISO 2001:3960; the findings were represented as meq of O2/kg of fat using the modified methods [21], the TBA was measured spectrophotometrically at 530 nm. The results were represented as mg MDA/Kg of fat. The TPC and RSA against to stable DPPH radical of lipid fraction estimated according methods in section 2.2.10

2.2.9. Organoleptic properties of the baked chocolate balls

Twenty trained panelists scored the baked balls covered in chocolate enriched with microencapsulated antioxidants on a 9-point hedonic scale regarding appearance, taste, flavor, crunchy, texture, sweetness, mouthfeel, and overall acceptability [22]. Each panelist has provided informed consent to take part in the research. The National Research Center's Food Technology Department laboratory conducted the organoleptic evaluation.

2.2.10. Diabetes induction

For inducing diabetes, rats were injected intraperitoneally with 45 mg/kg body weight (w/w) of streptozotocin (STZ) (from Sigma Chemical Co) and then given glucose solution (5%) for 48 h afterwards to prevent hypoglycaemia [23]. Glucose levels were measured 72 hours after streptozotocin injection to determine if rats had diabetes. Diabetic rats were those whose blood glucose level exceeded 200 mg/dl and were used for the following procedures.

2.2.11. Design of the animal study

Forty rats were divided into four groups of ten rats each. The first group was considered the normal healthy group (control normal). The remaining rats were served as diabetic rats. Diabetic rats were divided into three groups of ten each after developing diabetes:

- Group one: Untreated rats (control normal), were fed on the balanced diet.
- Group two: Diabetic rats were fed on the balanced diet.
- Group three: Diabetic rats were fed on the balanced diet supplemented with 20% of the prepared baked balls covered with dark chocolate without the micro-encapsulated synbiotic for 6 further weeks.
- Group four: Diabetic rats were fed on the balanced diet supplemented with 20% of the prepared baked balls covered with dark chocolate with the micro-encapsulated synbiotic for 6 further weeks.

During the experiment, body weight and food intake were recorded weekly. After forty five days (end of the study) total food intake, body weight gain and food efficiency ratio (Body weight gain/total food intake) were calculated. Samples of the rat's feces were collected after the induction of diabetes (zero time) and at the end of the feeding experiment (6 weeks) to determine the microbiological counts. Blood samples were collected from all rats after an overnight fast. The red blood cells

were analyzed for malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione and nitric oxide [24,25,26,27], respectively. Plasma was analyzed for tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), interleukin 6 (IL-6) and transforming growth factor β1 (TGF-β1) using Eliza kits (Sunlong Co., Ltd. China). Plasma also, was analyzed for fasting blood glucose levels [28], the activity of lactate dehydrogenase (LDH)[29], total cholesterol [30], high-density lipoprotein cholesterol (HDL-C) [31], low-density lipoprotein cholesterol (LDL-C) [32] and triglycerides [33]. Cholesterol/HDL-C ratio was calculated. The levels of creatinine and urea were determined [34], in succession [35]. The activities of aspartate transaminase (AST) and alanine transaminase (ALT) were determined [36]. This study has been approved by the Medical Research Ethics Committee, National Research Centre, Cairo, Egypt (No.17421122022).

2.2.12. The microbiological characteristics of rat's feces samples

The microbiological involved for rat feces, at the first day of feeding experiment and after 6 weeks of feeding, was determined using serial decimal dilution and pour plate method by different enumerated media as: Bifidobacterium sp. counts were enumerated on MRS agar complemented with 2gm/l sodium propionate and 3gm/l lithium chloride and the plates incubated anaerobically at 37℃/ 72h . Lactobacilli sp. counts were counted using MRS agar and the plates incubated anaerobically at 37°C / 72 h (IDF, 1997) and total bacterial counts were counted by plate count agar and incubated aerobically at 35°C/ 48h [37]. Coliform groups were detected using Violet Red bile Agar and the plates were incubated at 35°C/ 24 h. Mold and yeast counts were detected by acidified potato dextrose agar, pH 3.3 and the plates aerobically incubated for 25°C / 36h.

2.2.13. Statistical analysis

The results of animal experiments were expressed as the mean±SE and analyzed statistically using the one-way analysis of variance ANOVA followed by Duncan's test. In all cases $p<0.05$ was used as the criterion of statistical significance.

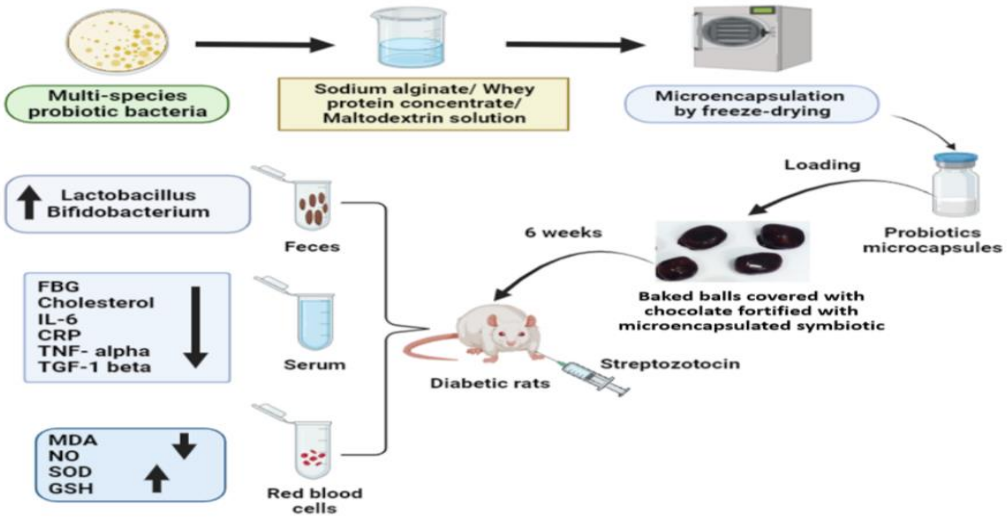

Figure 1 Application of probiotic cells pellets microcapsule for produce functional baked chocolate balls for prevalence type 1 diabetes.

3. Results and Discussion

3.1. Microencapsulation yield (% MY) of synbiotic microencapsulated powder

Microencapsulation technique is ideal for maintaining probiotic strain stability and viability under harsh environmental conditions, simplifying handling and allowing controlled dosage. It's crucial to determine probiotic counts inside capsules to ensure the encapsulation technique's suitability [38-40].

Additionally, it was necessary to confirm that the bacterial counts within the capsules above the critical threshold (more than 106 CFU/ml) in order for the probiotics to have a beneficial effect on the host upon ingestion (FAO/WHO, 2002) [41]. In this study, the technology of freeze-drying was employed to produce the powdered synbiotic capsules, with a documented

encapsulation yield of 91.15% for the powdered synbiotic microcapsules including a variety of probiotic strains and maltodextrin. Our findings were validated by other research, which showed that a high microencapsulation yield was linked to effective probiotic trapping within the microcapsules [42,43]. Furthermore, the numbers of probiotics were increased by the presence of a prebiotic agent [44,45].

3.2. Morphological pattern of synbiotic microencapsulated powder

The Morphological pattern for microencapsulated powder was determined using scanning electron microscope (SEM) as shown in Figure 2. From the images, the microcapsules were appeared in spherical shape in identical size around 70 µm, with smooth surface. Some accumulation with irregular and crumpled particles was observed. The probiotic strains not appeared in the SEM image, which good entrapped inside microcapsules and coated well with the materials (sodium alginate + whey protein). Thus, probiotics may be microencapsulated using freeze-dried technique, which produced tiny particles with distinct shapes. However, the low temperature causes ice crystals to develop, which could have an impact on the microcapsules' shape [46]. On the other hand, the high degree of aggregation and loss of spherical shape, resulting in a range of sizes for the microcapsules [47,48]. However, results showed that freeze-drier capsules had generally comparable beads with a smooth spherical surface [49].

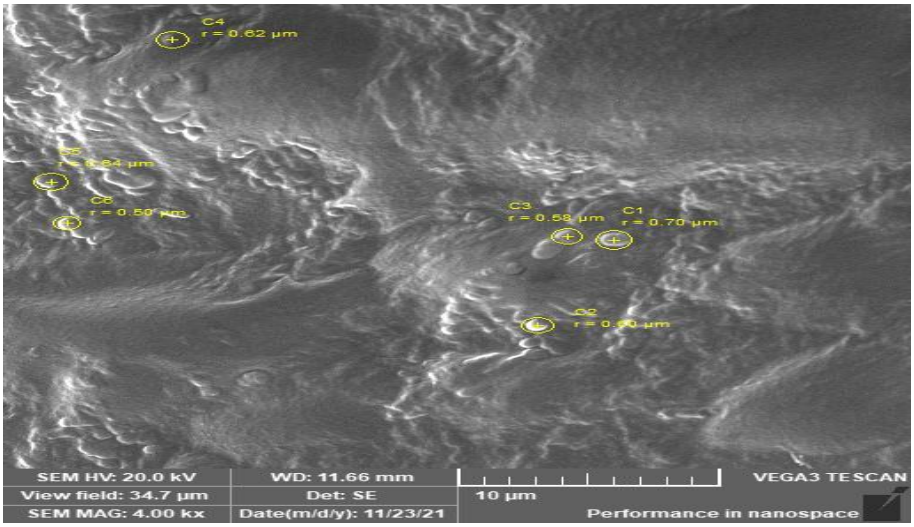

Figure 2. Morphological pattern for synbiotic microencapsulated powder using scanning electron microscope (SEM).

3.3. The viability of synbiotic microencapsulated powder during contact to stimulated gastrointestinal juices

The viability of probiotic strains either in encapsulated form or free cells were recorded in Figure 3. Initially, free and microcapsules probiotics were put in stimulated gastric juice (SGJ). The data observed decline in the viability of probiotics, but the more decrease was established for free cells. The bacterial counts were reduced around 1.00 and 1.70 log cycles for microcapsules and free cells, respectively. The encapsulation materials (sodium alginate+ whey protein concentrate) that used in this study were able to protect the cells than free after exposure to SGJ [50]. Also, presence the maltodextrin as prebiotic agent has positive impact on the probiotic viability [51,52]. Additionally, after being isolated from SGJ for an additional 6 hours, the still viable free and microcapsule cells were constantly added to intestinal juice (SIJ). In comparison to microcapsules, a quick log reduction was seen for unbound cells. After 6 hours, the viability in SIJ was measured to be 5.69 and 3.92 log CFU/g, with survival rates for microcapsules and free cells of 63.93% and 49.00%, respectively. The outcomes demonstrated the value of employing sodium alginate and whey protein materials to microencapsulate probiotic strains with prebiotic maltodextrin in order to preserve the viability of probiotic cells. According [53], modified starches with alginate using the freeze-drying method for encapsulation had a significant increase in initial viable cells compared to native starch after freeze-drying, and the probiotic bacteria were microencapsulated, which significantly improved acid tolerance.

According [54], introduction of Hi-Maize starch (a prebiotic) enhanced the encapsulation of live bacteria when compared to when the starch wasn't present.

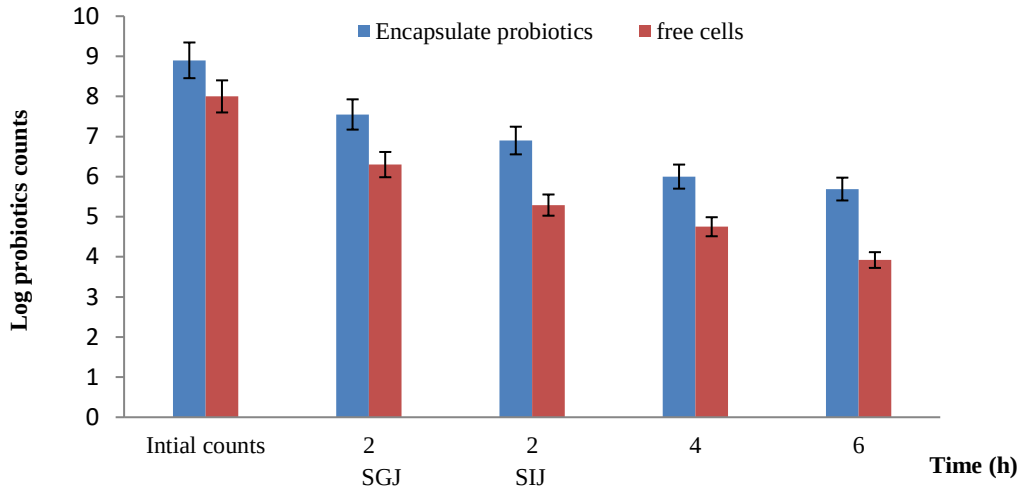

Figure 3. The viability of microencapsulated probiotic strains in simulated gastrointestinal conditions.

3.4. Macro and Micronutrients of prepared baked chocolate balls.

The proximate composition of baked balls enhanced with microencapsulated symbiotic bacteria was shown in Table 1 . The main ingredients of a chocolate ball are macronutrients such protein, lipids, ash, carbohydrates, and dietary fiber. In their most advantageous versions, each of these elements is present. Based on peanut butter, brown rice, eggs, and skim milk, the protein content was 13.66. the reduced risks of diabetes by a quarter when peanuts were incorporated in diet daily [55]. Table 1 also revealed that there were 13.80g of total dietary fiber and 354.2 kcal of calories. Reducing blood sugar and cholesterol levels with soluble fiber may help lower the risk of diabetes. Insoluble fiber increases insulin sensitivity in addition to intestinal health and regularity [56].

In terms of micronutrients, the minerals content of baked chocolate balls (Table 1) shows that the concentration of potassium, calcium, and phosphorus in chocolate balls is high, with respective values of 305.05, 345.2, and 454 mg/100g. A good source of vitamins, minerals, carotene, and other health-promoting compounds is pumpkin [57]. Peanut butter as a good source of magnesium that plays role against the development of diabetes were chosen to formulate the baked balls. The magnesium concentration of the chocolate ball samples was around 88.90 mg/100g. Magnesium, calcium, and potassium enhance peripheral glucose absorption, insulin sensitivity, and/or insulin secretion [58] . Essential micronutrients iron (Fe) and zinc (Zn) are involved in many physiological processes, including growth, development, and immunological function. Samples had 5.2 mg of iron and 4.7 mg of zinc per 100g;the egg and pumpkin in the recipe are responsible for the chocolate ball's zinc content. Zinc has antiviral properties and protects against β-cell death. According [59], there may be a connection between other nutritional deficiencies and the elevated incidence of anemia in people with diabetes. Pumpkin contained higher values of K, Fe and Zn(1592.0 , 41.50 and 15.21 mg/100g ,respectively [60].

Individuals diagnosed with diabetes were administered micronutrients, including vitamins A, E, and B complex [61]. Based on Table 1. findings about the micronutrients (A, E, and B complex) in the baked chocolate balls, one may infer that adding certain ingredients during the baking process would raise the amount of fat- and water-soluble vitamins. Vitamins A and E showed larger amounts of fat-soluble vitamins 36.00 µg and 3.81 mg. respectively, than other vitamins. Vitamin B6 and B12 levels in the samples were greater (1.40 mg and 24.01 µg/100g, respectively) for the B complex vitamins. The main way that vitamin E affects glucose regulation is by its strong lipophilic antioxidant activity. Other potential impacts include glycation of proteins, oxidation of fats, and production and sensitivity of insulin. Since vitamin B6 and B12 deficiency occurs in diabetic neuropathy patients, vitamins supplementation provides crucial protection against the development of diabetic neuropathy [62].

Table 1

Macro and Micronutrients of baked chocolate balls fortified with microencapsulated synbiotics

Macronutrients	**Amount**
Moisture (g/100g)	5.07
Ash (g/100g)	1.06
Protein (g/100g)	13.66
Fat (g/100g)	12.90
Carbohydrate (g/100g)	42.10
Energy (kcal)	354.2
Total dietary fiber (g/100g)	13.80
Soluble dietary fiber (g/100g)	1.70
Insoluble dietary fiber (g/100g)	12.10
Micronutrients	
Minerals:	
Potassium (mg/100g)	305.05
Calcium(mg/100g)	345.20
Magnesium(mg/100g)	88.90
Phosphorus(mg/100g)	454.10
Sodium (mg/100g)	270.30
Iron(mg/100g)	5.20
Zinc(mg/100g)	4.70
Vitamins:	
Vitamin A (µg/100g)	36.0
Vitamin E (mg/100g)	3.80
Vitamin B1 (mg/100g)	0.20
Vitamin B2 (mg/100g)	0.40
Vitamin B6 (mg/100g)	1.40
Folic acid B12 (µg/100g)	24.00

3.5. Antioxidant properties of baked chocolate balls

Antioxidants are effective by give up their own electrons to free radicals, leading to broken the chain reaction of oxidation [63]. Figure 4 presented the antioxidant properties of chocolate balls such as antioxidant activity, total phenolic and flavonoids contents.

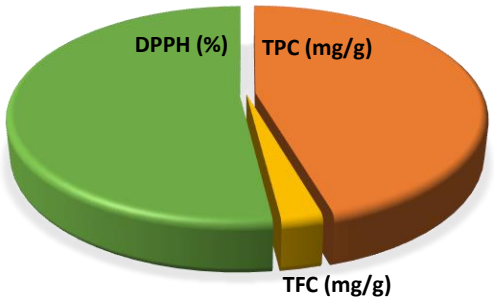

Figure 4. Antioxidant properties of baked balls covered with chocolate fortified with microencapsulated symbiotic

The results showed that chocolate balls contained high amount for TPC and TFC reached about 31.49 mg (GAE) and 1.72 mg (RE) /100g, respectively. While the antioxidant activity was found 36.01%. The highest content of polyphenols for pumpkin varieties was found ranged from 46.62 to 117.66 mg GAE/100g [64] . On the other hand, brown rice as a whole grain food is associated with various chronic diseases' reduced risks, the results indicated that the main components of brown rice with potent antioxidant activity were the total flavonoid content and phytosterols [65]. In the context, brown rice cultivars showed strong antioxidant activity and were abundant in phenolic compounds [66].

3.6. Oxidative stability of lipid fraction of chocolate balls during storage

Lipid oxidation is one of the important processes that occurs in most of the food systems and a major cause of food deterioration during processing and storage [67]. The oxidative stability of the chocolate ball's lipid components throughout storage was shown in Table (2). After 7 days, PV was higher (2.69meq/kg) than it was after 21 days (2.00meq/kg). The quality and composition of the study samples were significantly impacted by a two-month storage period at room temperature. After storage, peroxide values increased, but these parameters stayed within the CODEX Alimentarius [68], stipulated a permitted maximum peroxide level of not more than 10 milli equivalent of oxygen/kg of the oils. Measurement of peroxide greater than 10–20 meq/kg is often considered rancid. During storage period, TBA compounds displayed a little content rise.

The chocolate ball's thiobarbituric acid value (TBA) showed that the degradation rate decreased with a decrease in reaction rate constant and vice versa. After 21 days, DPPH and TPC were 62.66% and 16.91 mg GAE/kg oil, respectively, down from 77.55% and 17.31 mg GAE/kg oil at zero time. After three weeks of storage at room temperature, the peroxide value and TBA number were determined to be within a safe range, and the samples of chocolate balls showed no signs of deterioration. chocolate ball proves that ingredient choice intention was to be face with diabetes mellitus, healthy food product in terms of nutrient, antioxidant activity and energy.

Moreover, the predominant monounsaturated fatty acid found in peanuts and oleic acid have been primarily responsible for these health advantages [69]. Theseproduct have the potential to significantly improve the health of children with diabetes by acting as beneficial supplements and offering a novel method of consuming such nutritionally.

Table 2
Oxidative stability of lipid fractions of baked chocolate ball products at storage periods

Period/day	PV (meq/kg)	TBA (mgMDA/kg)	RSA (%)	TPC (mg GAE/Kg)
zero	2.56^{a}±0.05	0.93^{b}±0.03	17.31^{a}±0.16	77.55^{a}±0.94
7	2.68^{a}±0.05	1.06^{a}±0.04	16.95^{b}±0.28	70.35^{b}±0.26
14	2.03^{b}±0.02	1.06^{a}±0.03	16.97^{b}±0.16	66.17^{c}±0.74
21	2.00^{b}±0.03	1.06^{a}±0.04	16.91^{b}±0.25	62.66^{d}±1.83

Values are means ± SD. Means having the different case letter within a column are significantly different at $P \leq 0.05$.
Peroxide value (PV), Thiobarbituric acid (TBA), Radical scavenging activity (RSA), Total phenolic content (TPC).

3.7. Organoleptic Properties of the novel baked chocolate balls production

The baked balls covered with chocolate and enhanced with microencapsulated symbiotic and organoleptic qualities were depicted in Figure 5 and Table 3. Based on the sample's taste, flavor, crisp texture, sweetness, mouthfeel, and general acceptability, the ratings were calculated. The result in Table3, showed that the high sensory acceptability in all properties, this may be due to the unique combination of ingredients used in the product such as brown rice flour, peanut butter and pumpkin. A growing number of consumers now depended on pumpkins to be functional meals because of their high nutritional content and beneficial bioactive ingredients, in addition to its sensory acceptance [70]. The results demonstrated the highest ratings for flavor, sweetness, and mouthfeel, which inspired further investigation and analysis for children with diabetes who want sweets with superior phytochemical and sensory qualities.

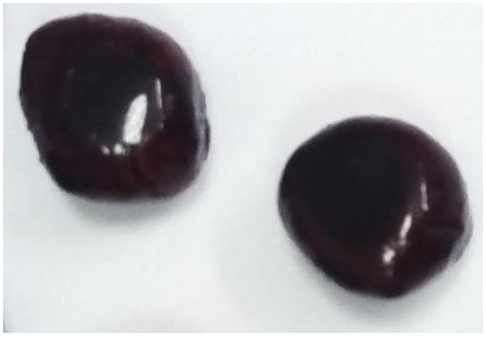

Figure 5. Novel baked chocolate balls production covered with chocolate fortified with microencapsulated symbiotic

Table 3
Organoleptic properties of the novel baked chocolate balls production

Organoleptic Properties	score
Appearance	8.70 ^{c} ±0.95
Taste	8.50 ^{d} ±1.27
Flavor	9.10 ^{b} ±0.57
Crunchy	8.20 ^{e} ±1.62
Sweetness	9.10 ^{b} ±0.88
Mouth feel	9.40 ^{a} ±0.70
Overall Acceptability	8.10 ^{f} ±0.88

Values are means ± SD. Means having the different case letter within a column are significantly different at P ≤ 0.05.

3.8. Effects of the prepared baked balls on diabetic rats.

The growth performance for different rat groups were showed in Table 4. The diabetic rats (Groups two) experienced considerably ($p \leq 0.05$) less body weight gain and a lower food efficiency ratio when comparison to the normal rats (Group one). Due to the catabolic process involved in diabetes, it has been established in earlier research that the introduction of diabetes reduced body weight gain and the food efficiency ratio [23,71]. Rats with diabetes that were fed the prepared baked balls with or without microencapsulated symbiotic (Group three, four) experienced significantly greater weight growth and food efficiency ratios ($p \leq 0.05$) than the diabetic group. Weight gain was considerably higher ($p \leq 0.05$) in diabetic rats fed the prepared baked balls containing microencapsulated synbiotics than in diabetic rats fed the prepared baked balls without microencapsulated synbiotic.

Table 4
Effect of the baked balls with or without microencapsulated synbioticon the growth performance

Growth performance parameters	Normal (Group one)	Diabetic (Group two)	Baked balls without the symbiotic (Group three)	Baked balls with the symbiotic (Group four)
Initial Body Weight (g)	147.60^{a}±4.82	147.50^{a}±2.17	147.30^{a}±2.88	147.40^{a}±3.39
Final Body Weight (g)	230.20^{d}±5.60	160.30^{a}±5.48	182.10^{b}±3.66	209.90^{c}±4.25
Body Weight Gain (g)	82.60^{d}±2.95	12.80^{a}±5.44	34.80^{b}±2.08	62.50^{c}±4.25
Total Food Intake (g)	685.00^{a}±3.79	700.00^{b}±3.05	687.00^{a}±3.58	693.00^{ab}±3.69
Food Efficiency Ratio	0.12^{d}±0.01	0.02^{a}±0.01	0.05^{b}±0.01	0.09^{c}±0.01
Relative Weight of Liver (%)	3.00^{a}±0.09	3.63^{b}±0.10	2.96^{a}±0.19	2.98^{a}±0.12
Relative Weight of Kidney (%)	0.72^{a}±0.03	0.79^{a}±0.04	0.79^{a}±0.04	0.80^{a}±0.05

A different superscript letter is tabulated in each row to signify a significant difference at P ≤ 0.05.

The anti-diabetic effect of baked balls is probably mainly due to its ingredients, especially pumpkin that scavenges ROS, reduces lipids and enhances the level of insulin in the body, leading to a lower level of glucose through the compounds it contains such as proteins, carotene, minerals, vitamins, polysaccharides, pectin, as well as phenolic compounds and terpenoids [72]. In baked balls with synbiotic microcapsules, plus ingredients, fortification with probiotics increases the anti-diabetic power of baked balls.

Table 5 shows that diabetic rats (Group two) recorded glucose concentration and TFG-β 1, TNF-α, CRP and IL-6 levels significantly ($p \leq 0.05$) higher than those of normal rats (Group one). Compared to the normal group, the diabetic group had significantly lower insulin level ($p \leq 0.05$). The production of free radicals like superoxide and hydroxyl radicals, which can quickly destroy pancreatic beta cells and lead to the development of hyperglycemia and accompanying consequences, is how STZ exerts its cytotoxic effect [73]. High amounts of bioactive molecules, such as superoxide free radicals, tumour necrosis factor, and interleukin-6, can be brought on by hyperglycemia [74]. Diabetic rats fed on balanced diets (Group three, four) supplemented with the prepared baked balls with or without microencapsulated synbiotic, significantly less for the prepared baked balls with microencapsulated synbiotic, recorded glucose concentration and TFG-β 1, TNF-α, CRP and IL-6 levels significantly less ($p \leq 0.05$) than those of diabetic group. Insulin level in diabetic rats fed on balanced diets supplemented with the prepared baked balls either with or without microencapsulated synbiotic was significantly ($p \leq 0.05$) higher than that of diabetic group.

The prepared baked balls with microencapsulated synbiotic were the most promising in elevation of insulin and reduction of glucose concentration and TFG-β 1, TNF-α, CRP and IL-6 levels. When the amount of probiotics increased in the intestine, the demand for glucose goes up resulting in a decrease in glucose in serum [75]. The hypothalamic-pituitary-adrenal (HPA) axis, which controls the pancreas and simultaneously modulates the release of glucagon and insulin, is impacted by the decrease in fasting cortisol levels brought on by probiotic therapy. Furthermore, it has been demonstrated that Bifidobacterium and Bacillus strains have an impact on the elevated expression of proteins implicated in the insulin signaling system [75]. Prebiotic and probiotic consumption has been shown to raise levels of anti-inflammatory cytokines while decreasing levels of pro-inflammatory cytokines, such as IL-6 IL-1β and TNF-α. Probiotics may therefore be useful in avoiding T1D [76].

Table 5

Effect of the baked balls with or without microencapsulated synbioticon glucose, insulin,TFG-β1, TNF-α and IL-6 levels among the studied four groups

	Normal (Group one)	**Diabetic (Group two)**	**Baked balls without the symbiotic (Group three)**	**Baked balls with the symbiotic (Group four)**
Glucose (mg/dl)	$89.17^{a}\pm2.72$	$299.70^{d}\pm5.28$	$128.10^{c}\pm2.46$	$99.76^{b}\pm1.21$
Insulin (mU/L)	$5.07^{d}\pm0.23$	$1.70^{a}\pm0.15$	$3.27^{b}\pm0.10$	$4.24^{c}\pm0.18$
TFG-β 1(ng/ml)	$23.08^{a}\pm0.46$	$77.19^{d}\pm0.84$	$52.20^{c}\pm0.59$	$37.05^{b}\pm0.71$
TNF-α (pg/ml)	$5.74^{a}\pm0.10$	$11.18^{d}\pm0.32$	$7.75^{c}\pm0.23$	$6.40^{b}\pm0.15$
CRP (ng/ml)	$2.86^{a}\pm0.07$	$5.47^{d}\pm0.11$	$4.58^{c}\pm0.10$	$3.78^{b}\pm0.16$
IL-6 (pg/ml)	$26.33^{a}\pm0.31$	$77.63^{d}\pm0.97$	$43.40^{c}\pm0.58$	$36.63^{b}\pm0.71$

A different superscript letter is tabulated in each row to signify a significant difference at $P \leq 0.05$.
Transforming growth factor β1 (TGF-β1), Tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), Interleukin 6 (IL-6).

Table 6 demonstrates that the reduced glutathione and superoxide dismutase (SOD) levels obtained by the diabetic rats (Group two) were significantly lower than those of normal rats (Group one). MDA and nitric oxide levels were significantly ($p \leq 0.05$) higher in the diabetic group than in the control normal group. ROS are known to be released to streptozotocin injection. Additionally, elevated ROS and decreased antioxidant enzymes are linked to prolonged hyperglycemia [71]. The reduced glutathione and SOD values of diabetic rats (Group three, four) fed the prepared baked balls, either with or without microencapsulated synbiotic, were considerably greater than those of the diabetic group. MDA and nitric oxide levels were considerably ($p \leq 0.05$) lower in diabetic rats fed the prepared baked balls, either with or without microencapsulated synbiotic. The prepared baked balls with microencapsulated synbiotic were the most promising in decreasing of MDA and nitric oxide values and increasing of reduced glutathione and SOD values. Probiotics inhibit the production of free radicals and nitric oxide [77].

Table 6
Effect of the baked balls with or without microencapsulated symbiotic on the oxidative markers

Oxidative markers	**Normal (Group one)**	**Diabetic (Group two)**	**Baked balls without the symbiotic (Group three)**	**Baked balls with the symbiotic (Group four)**
MDA (nmol/g Hb)	$9.13^{a}\pm0.15$	$19.03^{d}\pm0.35$	$13.52^{c}\pm0.20$	$10.94^{b}\pm0.54$
Reduced GSH (mg/g Hb)	$118.10^{d}\pm1.38$	$76.07^{a}\pm0.83$	$85.30^{b}\pm0.70$	$98.57^{c}\pm1.04$
NO (nmol/g Hb)	$1.37^{a}\pm0.05$	$3.89^{d}\pm0.05$	$2.41^{c}\pm0.07$	$1.73^{b}\pm0.07$
SOD (U/g Hb)	$156.53^{d}\pm1.81$	$93.75^{a}\pm0.82$	$121.37^{b}\pm0.80$	$137.80^{c}\pm0.92$

A different superscript letter is tabulated in each row to signify a significant difference at $P \leq 0.05$.
Malondialdehyde (MDA), Superoxide dismutase (SOD), Glutathione(GSH),Nitric oxide(NO).

From Table 7, it was found that the diabetic rats (Group two) had significantly higher values of total cholesterol, LDL-cholesterol, cholesterol to HDL ratio, and triglycerides than the normal rats (Group one). A significant ($p \leq 0.05$) difference was found between the HDL-Ch value of diabetics and that of the control group. There were significant differences in total cholesterol, LDL-cholesterol, Ch/HDL ratio, and triglyceride levels between the different diabetic rats (Group two, three and four). HDL-Ch value in diabetic rats fed on balanced diets supplemented with the prepared baked balls either with or without microencapsulated synbiotic was significantly ($p \leq 0.05$) higher than that of diabetic group. The prepared baked balls with microencapsulated synbiotic were the most promising in increasing of HDL-Ch value and decreasing of total cholesterol, LDL-Ch, Ch/HDL ratio and triglycerides values. The absence or the insufficient of the insulin lead to disturbance of the lipid metabolism resulting in dyslipidemia [78]. Probiotics may improve lipid metabolism either directly or indirectly by controlling insulin levels [78]. Moreover, the beneficial effect of baked balls on lipids can be attributed to their prebiotic content, mainly pectin found in pumpkin and maltodextrin used in encapsulations of probiotics [79].

Table 7
Effect of the baked balls with or without microencapsulated symbiotic on the lipid profile

lipid profile parameters	**Normal (Group one)**	**Diabetic (Group two)**	**Baked balls without the symbiotic (Group three)**	**Baked balls with the symbiotic (Group four)**
T. Cholesterol (mg/dl)	$102.69^{a}\pm1.41$	$158.17^{d}\pm1.74$	$128.92^{c}\pm1.05$	$107.91^{b}\pm2.64$
TG (mg/dl)	$77.05^{a}\pm1.77$	$150.44^{c}\pm2.74$	$102.59^{b}\pm2.12$	$83.57^{a}\pm2.69$
HDL-Ch (mg/dl)	$38.59^{c}\pm0.56$	$28.70^{a}\pm0.33$	$35.95^{b}\pm1.04$	$36.60^{bc}\pm0.91$
LDL-Ch (mg/dl)	$48.69^{a}\pm1.52$	$99.38^{c}\pm1.93$	$59.35^{b}\pm4.52$	$54.60^{ab}\pm3.23$
Ch/HDL ratio	$2.68^{a}\pm0.05$	$5.52^{c}\pm0.09$	$3.27^{b}\pm0.20$	$3.00^{ab}\pm0.13$

A different superscript letter is tabulated in each row to signify a significant difference at $P \leq 0.05$.
Triglycerides (TG), High-density lipoprotein cholesterol (HDL-Ch), Low-density lipoprotein cholesterol (LDL-Ch) Cholesterol/High-density lipoprotein (Ch/HDL).

Table 8 shows that diabetic rats (Group two) recorded AST, ALT, LDH, ALP, urea and creatinine values significantly ($p \leq 0.05$) higher than those of normal rats (Group one). Albumin and total protein values in diabetic group were significantly ($p \leq 0.05$) less than those of control normal group. Diabetic rats (Group three, four) fed on balanced diets supplemented with the prepared baked balls either with or without microencapsulated synbiotic recorded AST, ALT, LDH, ALP, urea and creatinine values significantly less ($p \leq 0.05$) than those of diabetic group. Albumin and total protein values in diabetic rats fed on balanced diets supplemented with the prepared baked balls either with or without microencapsulated synbiotic were significantly ($p \leq 0.05$) higher than those of diabetic group.

The prepared baked balls with microencapsulated synbiotic were the most promising in increasing of albumin and total protein values and decreasing of liver and kidney functions. Heperglycemia can lead to long-term damage, dysfunction and failure of some organs such as kidney and liver [80]. Administration of probiotics and prebiotics may stimulate the production of SCFAs, which boost the intestinal L-cells' ability to produce glucagon-like peptide-1 (GLP-1). A hormone called GLP-1 encourages the pancreatic beta cells to secrete more insulin, which lowers blood sugar levels [81]. As a result, the hypoglycemic action of synbiotic directly supports the preservation of liver and kidney function as well as the protection of tissues. Additionally, taking probiotics and prebiotics prevented the elevation of liver and kidney function [82].

Table 8

Effect of the baked balls with or without microencapsulated symbiotic on liver and kidney functions

liver and kidney functions	Normal (Group one)	Diabetic (Group two)	Baked balls without the symbiotic (Group three)	Baked balls with the symbiotic (Group four)
AST (U/l)	38.51^{a}±0.47	63.90^{d}±0.88	53.20^{c}±0.77	42.60^{b}±0.85
ALT (U/l)	28.00^{a}±0.52	43.20^{d}±0.85	36.60^{c}±1.45	31.50^{b}±0.72
LDH (U/l)	228.20^{a}±1.59	335.60^{d}±3.81	260.10^{c}±4.08	244.80^{b}±3.10
Urea (mg/dl)	27.96^{a}±0.86	36.65^{b}±0.83	30.03^{a}±1.45	28.05^{a}±0.92
Creatinine (mg/dl)	0.48^{a}±0.02	0.71^{c}±0.04	0.60^{b}±0.03	0.50^{a}±0.02
Albumin (g/dl)	4.71^{c}±0.33	2.59^{a}±0.13	3.53^{b}±0.15	4.30^{c}±0.17
T. Protein (g/dl)	7.14^{c}±0.13	5.82^{a}±0.14	6.48^{b}±0.11	6.90^{c}±0.11
Alkaline Phosphatase (U/l)	100.70^{a}±0.97	138.90^{d}±1.64	127.10^{c}±1.55	105.90^{b}±0.93

A different superscript letter is tabulated in each row to signify a significant difference at $P \leq 0.05$.
Aspartate transaminase (AST), Alanine transaminase (ALT), lactate dehydrogenase (LDH).

3.9. The microbiological characteristics of rat feces samples

The microbial counts of rats' feces before the intervention with the prepared balls (zero time) were recorded in Table 9. The counts of Bifidobacterium sp. was near in all rats' feces groups, where ranged between 5.68 and 6.24 log CFU/g. Also, the counts of lactobacilli sp. was near in all rats' feces groups and recorded between 5.70 and 6.62 log CFU/G, the more counts was observed in normal group samples. Additionally, the total bacterial counts were enumerated in all rats' feces groups and located between 5 and 6 log cycles. Also, the coliforms was detected and recorded in the same 5 log cycles for all groups. The same observation was recorded in the counts of mold and yeast in all rats' feces samples, where the counts detected between 4.61 and 5.12 log CFU/g. Diabetes has been associated with alterations in the gut microbiota, particularly with an increase in bacteriodes phylum and clostridium strain bacteria [83].

Table 9

Microbial strains counts of feces samples at zero time (Log CFU/g).

Microbial strains	Normal (Group one)	Diabetic (Group two)	Baked balls without the symbiotic (Group three)	Baked balls with the symbiotic (Group four)
Bifidobacterium sp.	6.24^{b} ± 0.22	5.68^{a} ± 0.18	6.04^{ab} ± 0.18	5.83^{ab} ± 0.12
Lactobacilli sp.	6.62^{b} ± 0.16	5.70^{a} ± 0.29	6.13^{ab} ± 0.16	5.83^{a} ± 0.20
Total bacterial counts	6.28^{b} ± 0.18	6.30^{b} ± 0.24	5.54^{a} ± 0.20	5.72^{ab} ± 0.16
Coliforms counts	5.21^{a} ± 0.09	5.59^{b} ± 0.14	5.10^{a} ± 0.13	5.75^{b} ± 0.13
Mold and yeast counts	4.61^{a} ± 0.16	4.65^{ab} ± 0.10	5.04^{bc} ± 0.16	5.12^{c} ± 0.13

A different superscript letter is tabulated in each row to signify a significant difference at $P \leq 0.05$.

The microbial counts of rats' feces after the intervention with prepared baked balls either with or without microencapsulated synbiotic were recorded in Table 10 we observed an increase in the counts of Bifidobacterium sp. in the rats' feces samples of group that feeding on the prepared baked balls with microencapsulated synbiotic, which recorded 8.17 log CFU/g. But the lowest Bifidobacterium sp. was recorded for rats' feces sample of diabetic group, which receded 5.02 log CFU/g. The same finding was observed for Lactobacilli sp. counts, where the more counts was recorded for rats' feces samples of group that feeding the prepared baked balls with microencapsulated synbiotic (8.32 log CFU/G), followed with the rats' feces samples of group feeding on the prepared baked balls without microencapsulated synbiotic (6.50 log CFU/g), and the low counts of Lactobacilli was recorded for rats' feces samples of diabetic group (4.95 log CFU/g). In contrast, the counts of coliforms, molds and yeasts were more in rats' feces samples of diabetic groups, where recorded 6.37and 5.33log CFU/g for coliforms, mods and yeasts, respectively and followed with the rats' feces samples of normal group. But the feeding on the prepared baked balls with microencapsulated synbiotic was gained the little counts for coliforms, mold and yeasts. This finding was confirmed the positive effect for the synbiotic on gut microbitoa of rats as probiotics were able to colonize inside

colon and competing with the harmful strains [84,85]. Also, the counts in the rats' feces samples of group fed on the prepared baked balls without microencapsulated synbiotic were significantly diminutive than rats' feces samples of diabetic group. The same finding was observed for total bacteria, where more counts were recorded for samples of diabetic group (6.30 log CFU/g) and near to the counts in sample for normal group (6.28 log CFU/g). It was established that feeding on the encapsulated synbiotics lead to a more healthy gut microbiota and enhanced the microbial population in the gastrointestinal tract [86-88].

Table 10

Microbial strains counts of feces samples after 6 weeks of feeding (Log CFU/g).

Microbial strains	Normal (Group one)	Diabetic (Group two)	Baked balls without the symbiotic (Group three)	Baked balls with the symbiotic (Group four)
Bifidobacterium sp.	$5.90^{b} \pm 0.16$	$5.02^{a} \pm 0.09$	$6.06^{b} \pm 0.19$	$8.17^{c} \pm 0.09$
Lactobacilli sp.	$6.17^{b} \pm 0.15$	$4.95^{a} \pm 0.20$	$6.50^{b} \pm 0.17$	$8.32^{c} \pm 0.11$
Total bacterial counts	$6.66^{c} \pm 0.20$	$7.03^{c} \pm 0.15$	$5.31^{b} \pm 0.17$	$4.34^{a} \pm 0.08$
Coliforms counts	$5.20^{b} \pm 0.20$	$6.37^{c} \pm 0.11$	$4.89^{b} \pm 0.19$	$3.87^{a} \pm 0.15$
Mold and yeast counts	$4.80^{b} \pm 0.15$	$5.33^{c} \pm 0.16$	$4.62^{b} \pm 0.16$	$3.49^{a} \pm 0.13$

A different superscript letter is tabulated in each row to signify a significant difference at $P \leq 0.05$.

4. Conclusion

It was apparent that the prepared baked chocolate balls containing micro-encapsulated synbiotics had antioxidant power, reduced blood glucose levels, and increased insulin levels in diabetic rats. Furthermore, in diabetic rats, the prepared balls reduced the increase of malondialdehyde, nitric oxide, TNF-α, C-reactive protein, IL-6, and transforming growth factor β1 and the inflammatory markers. The prepared balls influenced the gut microbiota by increasing Bifidobacterium and Lactobacillus content. Consuming the baked balls appeared to be a viable substitute for reducing blood sugar and averting problems from diabetes or diabetic complications. It was clear that the prepared baked chocolate balls with micro-encapsulated synbiotics contained ingredients like brown rice flour, peanut butter, and fresh pumpkin, which give the product antioxidant activity (DPPH and TPC content) and no signs of deterioration (TBA and Peroxide value) after three weeks of storage, it was prove that the product was healthy, affordable, and nutritious.

5. Conflicts of interest : The authors declare no conflict of interest

6. Formatting of funding sources

The authors gratefully acknowledge the support of Food Technology, Nutrition and Food Sciences and Dairy Departments, National Research Centre, Dokki, Cairo, Egypt

7. Compliance with ethics requirements:

The Medical Research Ethics Committee, National Research Centre, Cairo, Egypt, approved the animal experiment on ethical grounds (No.17421122022).

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