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The Potential Impact of Different Types of Yogurt Fortified with Inulin and/or Microencapsulated Probiotic Bacteria on Diabetic Rats

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Abstract

Diabetes is a metabolic disease that is very common in developing countries and responsible for the death of over 1.5 million people each year. This study aimed to investigate the anti-diabetic effect of different types of synbiotic yogurt on experimental rats. Four different types of synbiotic yogurt supplemented with inulin and microencapsulated probiotics (*Lactobacillus acidophilus* CH2, *Lactobacillus plantarum* DSA 20174, *Lactobacillus rhamnosus* NRRL B-442, *Bifidobacterium lactis* LB-12) were used. A fifth type of yogurt supplemented with inulin alone was also prepared. The anti-diabetic effect of different synbiotic yogurt was evaluated in diabetic rats. The results showed that the microencapsulation had improved the survival of probiotic in yogurt samples. The yogurt samples supplemented with inulin and microencapsulated *L. acidophilus* have a mean viability (8.6 ±0.1 log CFU/g) on the seventh day higher, but not significant, than the viability at zero time (8.1 ±0.1 log CFU/g). The glucose and total cholesterol levels have significantly decreased (132 mg/dL and 72 ±5 mg/dL at $P<0.05$ respectively) in diabetic rats that were fed with yogurt supplemented with inulin and *L. acidophilus* compared to the diabetic control group (glucose 360 mg/dL and total cholesterol 118 ±4 mg/dL). The administration of yogurt supplemented with inulin and *L. rhamnosus* and yogurt supplemented with inulin and *B. lactis* were the most promising in improving plasma ALT (26 ±1 U/L) and AST (32 ±1 U/L) levels respectively, compared to those of diabetic control group (ALT 127 ±4 U/L and AST 69 ±0.8 U/L). A significant reduction ($P<0.05$) was also recorded in the levels of creatinine (0.75 ±0.09 mg/dL) and urea (30 ±0.4 mg/dL) in diabetic rats that were fed yogurt supplemented with inulin and *L. plantarum* compared to that of the diabetic control group (creatinine 3.08 ±0.07 mg/dL and urea 72 ±2 mg/dL). In general, the results in the current study provided evidence that using the microencapsulation technique can enhance the viability and the performance of the probiotic bacteria. The results also support the application of probiotic bacteria in ameliorating type-2 diabetes and reducing its complications.

Keywords: Probiotics, Synbiotic, Microencapsulation, Yogurt, Diabetic rats.

1. Introduction

Type-2 diabetes mellitus, a metabolic disorder described by hyperglycemia, is being attributed to different physiological, genetic, and environmental factors (Hofe *et al*., 2014). However, the main cause of type-2 diabetes is a result of the deficiency in insulin secretion or insulin action due to the dysfunction of islet B-cell (Asemi *et al*., 2013). A series of morphological and functional alterafftions occur during diabetes mellitus, which can trigger some complications (Ajiboye *et al*., 2018). Recently, the gut microbiota received considerable attention by nutritionists due to its interesting function in controlling the insulin level (Brunkwall and Orho-Melander, 2017; Samanta *et al*., 2018). Probiotic bacteria are living microorganisms present naturally in human and animal gut and have different beneficial effects (Karim and Hasan, 2019). For instance, they contribute in synthesizing of vitamin and antimicrobial compounds (Karim and Hasan, 2019; Alrabadi *et al*., 2018), boosting the immune system, reducing cholesterol (AL-Awwad *et al*., 2014), and use in cancer therapeutic application (Vijayaram and Kannan, 2018). Moreover, the role of gut microbiota in metabolic diseases, including type-2 diabetes, became evident (Gurung *et al*., 2020; Bera and Ghosh, 2018).

Lactic acid bacteria (LAB), probiotic bacteria which are common in our natural environment, are being commonly used in dairy industries across the world for thousands of years (Evivie *et al*., 2017). The presence of some *Lactobacillus* strains in human gut flora, in addition to its long history of use in foods and dairy products without significant complications, has led to the conclusion that they are safe for human consumption (Jones *et al*., 2012; Mahasneh and Abbas, 2010). Nowadays, probiotic bacteria are being widely used in many functional foods

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and also in treating many physiological and metabolic diseases (Jones *et al*., 2012). Prebiotics are non-digestible oligosaccharides that are enhancing the growth of beneficial commensal organisms (Ooi *et al*., 2010).

Yogurt is the most popular dairy product that contains probiotic bacteria, and is widely consumed due to its high nutritional benefits (Suliman and El Zubeir, 2014). As such, yogurt is regarded as an ideal form for the successful delivery of probiotic bacteria (Sanders, 2008). There are some factors (e.g. acidity, level of oxygen in products, presence of other lactic acid bacteria, and byproduct produced by other competing bacteria) that might negatively affect the shelf life of probiotic supplemented products. These factors could, individually or collectively, impairer the efficacy of probiotic bacteria (Terpou *et al*., 2019). Recently microencapsulation of probiotic bacteria has been used to enhance the efficiency of functional foods (De Prisco and Mauriello, 2016). The technique of microencapsulation has been proven to enhance the viability of some sensitive microorganisms against the harsh environmental conditions (Huq *et al*., 2013). This study aimed to manufacture different yogurt samples containing inulin and microencapsulated probiotic bacteria and investigates their anti-diabetic effects on diabetic rats.

2. Materials and Methods

2.1. Preparation of Microorganism

The probiotic bacteria were obtained from different sources. For instance, *Lactobacillus acidophilus* CH2 and *Lactobacillus rhamnosus* NRRL B-442 were purchased from Chr. Hansen's Lab., DENMARK and the Northern Regional Research Laboratory, ILLINOIS, USA, respectively. *Lactobacillus plantarum* DSA 20174 and *Bifidobacterium lactis* LB-12 were purchased from MIRCEN, FACULTY OF AGRICULTURE, AIN SHAMS UNIVERSITY, EGYPT.

All *Lactobacillus* strains, (*L. acidophilus, L. plantarum,* and *L. rhamnosus*) were grown on MRS broth (purchased from SRL, INDIA and sterilized for 15 min at 120ºC) and incubated at 37^{o}C for 24 hours (De Man *et al.,* 1960), while *Bifidobacterium* strain was grown on modified MRS broth (MRS enriched with L-cysteine hydrochloride 0.05%) to provide anaerobic conditions using the GasPak system (Collins and Hall, 1984; Vinderola and Reinheimer, 1999).

2.2. Microencapsulation of Bacterial Strains

The microencapsulation was prepared using the extrusion technique (Nigam *et al*., 1988) that was modified by Sharaf *et al* (2016)**.** The strain biomass was obtained by performing 4000 rpm centrifugation at 4°C for 15 minutes and then rinsed with 0.1% (w/v) sterile peptone (purchased from BACTO, DIFCO Laboratory, USA). The pellets were suspended in 5 ml of 0.1% (w/v) peptone and mixed with the same amount of 2% (w/v) sodium alginate solutions (purchased from JUDEX laboratory, ENGLAND and sterilized at 121°C for 15 minutes). The mixtures were dropped into a sterile $CaCl_2$ solution through a needle with gently stirring, forming beads to entrap the bacterial cells. The beads (2 mm-diameters) were washed twice in sterile saline to discard free cells and the remains of calcium ions. Finally, beads were washed with 0.1% sterile peptone solution and kept in peptone solution at 4°C. The entrapped bacteria were released from the capsules using 1g of the microcapsules dissolved in 9 ml of 2% sterile tri-sodium citrate solution and vortexed till complete dissociation (Zhou *et al*., 1998). The viability of probiotic bacteria inside the microcapsules was estimated using the pour-plate method (Vinderola and Reinheimer, 1999)**;** the results were recorded as colony-forming units for each gram (CFU/g).

2.3. Yogurt Preparation

A control set yogurt was prepared with yogurt starter culture only, without inulin and probiotic cultures. Five batches of set yogurt were prepared and supplemented with inulin and probiotic bacterial cultures except one batch (T5) was prepared and supplemented with inulin only. The yogurt samples were prepared by heating standardized buffalo milk (purchased from the local market) to 85°C for 30 minutes before cooling it to 37°C. Then, the milk was inoculated with a mixture of 3% inulin (purchased from El-SHARQ El-AWSAT company, CAIRO, EGYPT), 2% of the prepared microencapsulated probiotic culture, and 2% of yogurt starter culture, which contained *L. delbruekii bulgaricus* and *Streptococcus thermophilus* (Lee and Lucey, 2010). The probiotic bacteria with the yogurt were incorporated with the yogurt either as microencapsulated cells or free cells. The cultures of probiotic bacteria were added at the same time with the yogurt starter cultures. The inoculated milk was distributed in 100 ml plastic cups and then incubated at 37ºC. After that, the yogurt was cooled and stored at 4ºC for seven days. The chemical and microbiological analyses were carried out at 'zero' time (after overnight cold storage of samples) and repeated at the third day and seventh day of storage (Kailasapathy, 2006).

2.4. Analysis of Different Synbiotic Yogurt contents

2.4.1. Chemical Analysis

The pH of yogurt samples was determined using a pH meter (AD1000 pH /mV and temperature meter, Adwa Instruments), which was calibrated with reference buffer solutions (pH 4.0 and pH 7.0). All samples of synbiotic yogurt were stirred well before measuring the pH level (Kailasapathy, 2006). The moisture, ash, protein, and fat contents of the samples were determined after 24 hours of product storage at 4°C. For moisture content, a sample of fresh yogurt was accurately weighed and dried in oven at 105°C until reached a constant weight (Bradley, 2010). Ash content was determined by heating the yogurt sample at 550°C in a muffle furnace chamber (Marshall, 2010). The crude protein content was measured using Kjeldahel method (the total nitrogen was multiplied by a factor of 6.25) (Chang, 2010). Fat content was determined following Gerber method (Min and Ellefson, 2010). Total carbohydrate was calculated based on the amount of protein, ash and lipid using the following equation:

Total carbohydrate = 100(dry weight) - (protein + lipid + ash) (BeMiller, 2010).

2.4.2. Microbiological Analysis of Different Synbiotic Yogurt

A 10 g of synbiotic yogurt dissolved in 90 ml of 2% sterile citrate buffer. The selective media were used to differentiate the introduced microencapsulated probiotic

cultures from yogurt starter cultures. Both *L. acidophilus* and *L. plantarum* were detected using a selective media of MRS broth—MRS broth supplemented with maltose instead of glucose—and incubated at 37°C for 24 hours. *B. lactis* was grown in MRS broth enriched with L-cysteine hydrochloride 0.05% to allow anaerobic conditions (Collins and Hall, 1984). *L. rhamnosus* was inoculated into MRS supplemented with vancomycin antibiotic, where 0.005 g vancomycin dissolved in10 ml sterile distilled water, then 0.2 ml of this dissolved antibiotic solution was added to 100 ml MRS broth media at 55ºC (Tharmaraj and Shah, 2003).

2.5. Biological Evaluation of Different Synbiotic Yogurt

A total of 42 male rats (Sprague-Dawley), each with an average weight of 110 ±5 g, were obtained from the animal house of the NATIONAL RESEARCH CENTER, CAIRO, EGYPT. The animal's house ethics committee, NRC, GIZA, EGYPT, approved the safety and ethics. Rats were kept individually in stainless steel wire bottom cages at room temperature (25 ±2ºC) under 12 hours dark and light cycle. Animals were fed with stock diets ad-lib for three weeks until rats weighing 200 ±10 g. Next, rats have been induced to diabetes disease by injecting them intraperitoneally, while they were fasting, with 5% alloxan solution (135 mg/kg body weight) in saline solution (Federiuk *et al*., 2004). Blood samples were obtained from the tail vein to test the fasting blood glucose levels. On the seventh day after alloxan injection, rats with a level of blood glucose above 200 mg/dL were included in the study as diabetic rats. The rats were distributed into seven groups (each included six rats) where each group fed with balanced diet supplemented with different synbiotic yogurt as shown in (Table 1).

Table 1. Experimental groups

Groups	Diet
Control normal	Normal rats fed with a balanced diet*
Control diabetes	Diabetes rats fed with a balanced diet
Group (1)	Diabetic rats fed with a balanced diet and synbiotic yogurt have 3% inulin with 2% microencapsulated *L. acidophilus*
Group (2)	Diabetic rats fed with a balanced diet and synbiotic yogurt have 3% inulin with 2% microencapsulated *L. plantarum*
Group (3)	Diabetic rats fed with a balanced diet and synbiotic yogurt have 3% inulin with 2% microencapsulated *L. rhamnosus*
Group (4)	Diabetic rats fed with a balanced diet and synbiotic yogurt have 3% inulin with 2% microencapsulated *B. lactis*
Group (5)	Diabetic rats fed with a balanced diet and synbiotic yogurt have 3% inulin only

*Balanced diet is a diet that consists of 12% casein, 15% corn oil, 10% sucrose, 3% fiber, 55.5% starch, 3.5% salt mixture, and 1% vitamin mixture has been prepared for rats feeding during the period of the experiment (Reeves *et al*., 1993).

After five weeks of administration of different synbiotic yogurt, rats were forced to overnight fasting before collecting blood samples to measure the level of fasting blood glucose and HbA1c following Trinder (1969) and Trivelli *et al* (1971) respectively. The lipid profile of experimental rats was assessed by determining the levels of: (1) total cholesterol (T-CH) according to Allain *et al* (1974), (2) triglycerides (TG) (Fossati and Prencipe, 1982), (3) high-density lipoprotein cholesterol (HDL-CH) (Burstein *et al*., 1970), and (4) low-density lipoprotein cholesterol (LDL–CH) (Wieland and Seidel, 1983). The liver performance was evaluated by determining the activity of liver enzymes, specifically alanine transaminase (ALT) and aspartate transaminase (AST) (Reitman and Frankel, 1957). To assess changes in kidney functions and performances, the levels of creatinine and urea were evaluated according to Fawcett and Scott (1960) and Bartels *et al*. (1972) respectively.

2.6. Statistical Analysis

Analysis of Variance (ANOVA) with post-hoc Least significant difference (LSD) test was applied to assess the variability between the treatments and the controls (Waller and Duncan, 1969). All statistical analyses were carried out using IBM SPSS Statistics (IBM, 2019).

3. Results and Discussion

3.1. Viability of Probiotic Bacteria inside the Microcapsule

The viable counts of probiotic bacteria within calcium alginate microcapsule are shown in (Table 2). All strains reached 10^9 CFU/g of their viable counts inside the microcapsule. There was no significant ($P>0.05$) difference in the viability between the strains. Previous studies suggested a minimum viability threshold of 10^6 CFU/g to achieve the therapeutic effects from the probiotic bacteria (Dave and Shah, 1997), while a viable count above 10^7 would maximize the therapeutic effects from the probiotics (Lourens-Hattingh and Viljoen, 2001). These indicate that the viability of the probiotics in our study have high beneficial health properties.

Table 2. Viable counts of probiotic bacteria inside the microcapsule (log CFU/g)

Type	Bacterial count
L. acidophilus	8.64 ±0.3 [a]
L. plantarum	8.69 ±0.5 [a]
L. rhamnosus	8.74 ±0.5 [a]
B. lactis	9.03 ±0.6 [a]

Each value represents the mean ± SE Means followed by the same letter are not significantly different ($P>0.05$)

3.2. Analysis of Different Synbiotic Yogurt contents

3.2.1. Chemical Analysis of Different Synbiotic Yogurt

Data displayed in (Table 3) represent the pH values of the yogurt supplemented with microencapsulated probiotic cells, and yogurt supplemented with free probiotic. Our results showed that the pH value of yogurt supplemented with microencapsulated cells at the third and seventh days of storage was less than that of yogurt supplemented with free cells of probiotic bacteria. This decrease in pH value may be contributed positively to microencapsulated cell metabolism.

Table 3. pH of yogurt supplemented with free and microencapsulated probiotic bacteria

Yogurt types	Free probiotic cells			Microencapsulated cells		
	Zero time	Third day	Seventh day	Zero time	Third day	Seventh day
Control*	4.95	4.48	4.42	4.95	4.48	4.42
T1	4.99	4.78	4.53	4.54	4.24	3.95
T2	4.99	4.85	4.43	5.06	4.50	4.14
T3	4.99	4.66	4.52	5.07	4.52	4.24
T4	4.78	4.78	4.46	5.10	4.56	4.33
T5*	5.45	5.10	4.94	5.45	5.10	4.94

*Control yogurt was the same in the case of free cells and microencapsulated cells

Treatment 1 (T1): yogurt supplemented with 3% inulin with 2% microencapsulated *L. acidophilus* . Treatment 2 (T2): yogurt supplemented with 3% inulin with 2% microencapsulated *L. plantarum* Treatment 3 (T3): yogurt supplemented with 3% inulin with 2% microencapsulated *L. rhamnosus* Treatment 4 (T4): yogurt supplemented with 3% inulin with 2% microencapsulated *B. lactis* *Treatment5 (T5): yogurt supplemented with 3% inulin only, was the same in case of free cells and microencapsulated cells

This finding is congruent with that of Afzaal *et al* (2018), who reported a significant decrease in the pH value of the yogurt supplemented with inulin and microencapsulated lactic acid bacterial cells compared with yogurt supplemented with inulin and free cells of lactic acid bacteria. Sultana *et al*. (2000) attributed this decrease in the pH value to the gradual uptake of nutrients and the slow release of metabolites across the shell of microencapsulated alginate beads.

The moisture, protein, and fat of control buffalo yogurt were significantly higher than those of the other treatments (Table 4). However, there was no significant differences (*P=0.086*) between ash content of the control sample and that of the other treatments (Table 4). This finding is incongruence with that obtained by Stijepić *et al*. (2013), who reported high ash content (0.739% w/w) in yogurt supplemented with 3% inulin. This disagreement with our finding because Stijepić *et al*. (2013) have used yogurt prepared from cow milk, not buffalo. Furthermore, it is known that the ash content depends on the food supplement and also varies between seasons (Rasheed *et al*., 2016; Barlowska *et al*., 2011). This is another possible explanation for the disagreement between our finding and finding of Stijepić *et al*. (2013). Our result showed that the carbohydrates content of the control sample was significantly lower than those of other treatments. Previous study showed that carbohydrates can provide an appropriate media for the growth of beneficial bacteria, which, in turn, enhances gastrointestinal health and many physiochemical processes (Chandran *et al*., 2016). Therefore, the high the carbohydrate contents, the more health benefit. Interestingly, the moisture content value of the control yogurt in our study is in agreement with the moisture content value (86.40%) reported in a previous study (Hassan and Amjad, 2010).

Table 4. Chemical analysis of different synbiotic yogurt (% dry weight)

Treatments	Moisture	Ash/(DM *)	Protein/(DM *)	Fat/(DM *)	Carbohydrate/(DM*)
Control	86 ±0.9 ^{a}	3.5 ±0.4 ^{a}	24.7 ±0.1 ^{a}	41.8 ±0.1 ^{a}	30 ±1.1 ^{b}
T1	80.7 ±0.03 ^{b}	2.9 ±0.1 ^{a}	21±0.03 ^{b}	36 ±1.9 ^{b}	40.1±2.5 ^{a}
T2	80.7 ±0.05 ^{b}	3 ±0.1 ^{a}	20.3 ±0.3 ^{b}	36.9 ±0.3 ^{b}	39.8 ±0.1 ^{a}
T3	80.7 ±0.3 ^{b}	2.8 ±0.08 ^{a}	21±0.6 ^{b}	37 ±0.8 ^{b}	39.2 ±2.2 ^{a}
T4	80.7 ±0.6 ^{b}	3 ±0.1^{a}	20.6 ±0.09 ^{b}	36.5 ±0.7 ^{b}	40.9 ±1.3 ^{a}
T5	80.7 ±0.5 ^{b}	2.8 ±0.02 ^{a}	21.1 ±0.9 ^{b}	35 ±0.4 ^{b}	41.1±1.1 ^{a}

Each value represents the mean ± SE.
*DM means dry matter.
Means in the same column followed by the same letter were not significantly different (*P>0.05*). Control yogurt contains no inulin and no microencapsulated probiotic bacteria
Treatment 1 (T1): yogurt supplemented with 3% inulin with 2% microencapsulated *L. acidophilus.* Treatment 2 (T2): yogurt supplemented with 3% inulin with 2% microencapsulated *L. plantarum.* Treatment 3 (T3): yogurt supplemented with 3% inulin with 2% microencapsulated *L. rhamnosus*. Treatment 4 (T4): yogurt supplemented with 3% inulin with 2% microencapsulated *B. lactis.* Treatment 5 (T5): yogurt supplemented with 3% inulin only.
Carbohydrate was calculated not evaluated

Moreover, our result is also incongruence with Rinaldoni *et al*. (2012) who reported low fat content (15g/L) and high protein content (59g/L) in the yogurt sample supplemented with 3% inulin only compared with our results. This incongruence could be because the yogurt samples in Rinaldoni's study were prepared from a different source of milk, soymilk. The content of protein in synbiotic yogurt varies according to the proteolytic activity of the probiotics, which converts the protein into its functional units, peptides and amino acids (Hassan and Amjad, 2010).

3.2.2. Microbiological Analysis of Different Synbiotic Yogurt during the Storage Period

The differences in viable counts of microencapsulated strains in synbiotic yogurt during storage periods at the refrigerator are shown in (Table 5). All treatments have

shown the appropriate growth of microencapsulated strains. The viable counts of all treatments have increased until the seventh day. The overall means across storage periods for all treatments indicated that the viable counts reached the highest value on the third day of storage (e.g. samples supplemented with inulin and microencapsulated *L. acidophilus*). This increase reflects the protective effect of microencapsulation on the viability of strains. Samples supplemented with inulin and microencapsulated *L. plantarum* have a significant decrease at the seventh day of storage (8.2 ±0.2, *P=0.02*) compared to those of the third day of storage.

Table 5. Total viable bacterial counts in synbiotic yogurt during the storage period (log CFU/g)

	Microencapsulated cells		
Treatments	Zero time	Third days	Seventh days
T1	8.1 ±0.1 [a]	8.7 ±0.2 [a]	8.6 ±0.1 [a]
T2	8.2 ±0.1 [b]	8.4 ±0.06 [ab]	8.2 ±0.2 [c]
T3	8.1 ±0.09 [a]	8.8 ±0.2 [a]	8.3 ±0.2 [a]
T4	8 ±0.3 [a]	8.5 ±0.1 [a]	8.3 ±0.2 [a]

Each value represents the mean ± SE.

Means in the same row followed by the same letter are not significantly different (*P>0.05*)

Treatment 1 (T1): yogurt supplemented with 3% inulin with 2% microencapsulated *L. acidophilus* 'Treatment 2 (T2): yogurt supplemented with 3% inulin with 2% microencapsulated *L. plantarum* .Treatment 3 (T3): yogurt supplemented with 3% inulin with 2% microencapsulated *L. rhamnosus*. Treatment 4 (T4): yogurt supplemented with 3% inulin with 2% microencapsulated *B. lactis*

Our results are in agreement with Brinques and Ayub (2011), who reported high viability for microencapsulated bacteria compared to the viability for free cells. This variation in viability is a result of the effectiveness of microencapsulation in maintaining the stability of the probiotic bacteria under storage at refrigeration temperature (Brinques and Ayub, 2011). Pavunc *et al* (2011) also found a better growth and high survival rate for *L. helveticus* M92 inside the microcapsule compared to free cells during yogurt fermentation. This growth pattern could be a result of the bidirectional diffusion of nutrients and metabolites through pores of the microcapsule (Pavunc *et al*., 2011). The reported decrease in the growth of probiotic at the seventh day in the current study was expected because the accumulation of undissociated acids inside the microcapsule, which, in turn, leads to decrease in the growth and the biomass of the probiotics (Klinkenberg *et al*., 2001).

3.3. Biological Evaluation of Different Synbiotic Yogurt

3.3.1. Anti-diabetic Effect of Different Synbiotic Yogurt

The results of the current study showed that consuming yogurt supplemented with synbiotic could decrease the level of fasting blood glucose and glycosylated hemoglobin of diabetic rats (Table 6). At the end of the experiment, a significant reduction in the level of blood glucose (*P< 0.001*) and HbA1c (*P< 0.021*) was reported in all groups. However, the rats in group 1 that were fed with a balanced diet supplemented with inulin and microencapsulated *L. acidophilus* showed the highest significant reduction in the level of blood glucose (132 ±3 mg/dL, *P= 0.001*).

Table 3. Anti-diabetic diet effect on plasma glucose and HbA1c of different experimental rats.

Group	Plasma glucose (mg/dL)	HbA1c %
Control normal	66 ±1 [e]	<4 ±0 [d]
Control diabetes	360 ±2 [a]	8 ±0.03 [a]
Group (1)	132 ±3 [d]	4 ±0.27 [cd]
Group (2)	144 ±3 [c]	4 ±0.16 [cd]
Group (3)	136 ±5 [cd]	6 ±0.63 [b]
Group (4)	182 ±4 [b]	5 ±0.40 [bc]
Group (5)	185 ±5 [b]	5 ±0.50 [bc]

Each value represents the mean ± SE. In each column, the same letters mean no significant difference at *P<0.05*.

It has been suggested that the decrease in the level of blood glucose in diabetic or non-diabetic people is due to the consumption of probiotic bacteria or synbiotic (Nikbakht *et al*., 2018). Probiotic bacteria play an important role in gut flora modification, which stimulates glucose absorption by producing insulin-tropic polypeptide and glycogen–link peptide (Nikbakht *et al*., 2018). Another study has also reported that the probiotic strains *MTCC 5690* and *MTCC 5689* have decreased the blood glucose level (131 mg/dL and 129 mg/dL), respectively, compared to the diabetic group (167 mg/dL) (Balakumar *et al*., 2018). This decrease attributed to the ability of the probiotic strains to improve the gut integrity, decrease LPS (Lipopolysaccharide), and increase GLP-1 (Glucagon-like peptide-1), which, subsequently, enhances insulin sensitivity (Balakumar *et al*., 2018).

Probiotic can also indirectly reduce the glucose level by: 1) changing the activities of the autonomic nerve, which, in turn, reduces the secretion of glucagon (Yamano *et al*., 2006), and 2) enhancing the antioxidant status of diabetic patient, which, in turn, prevents the destruction of β-cells and decreases the oxidative damage (Zhang *et al*., 2016). Furthermore, probiotic also has the potential to inhibit the absorption of glucose in the intestine, which leads to a reduction in the glucose level (Zhang *et al*., 2016).

Previous studies could not find significant differences between synbiotic yogurt supplemented with probiotics and conventional yogurt on the glucose levels in patients with diabetes or obesity (Barengolts *et al*., 2019). These results can be attributed to: 1) using small sample size, 2) sub-therapeutic doses, and 3) short duration for the experiments, which negatively affects the accuracy of the statistical analyses and the final conclusion (Mazloom *et al*., 2013).

3.3.2. Effect of Different Synbiotic Yogurt on Plasma Lipid Profile

Data in (Table 7) represent the plasma lipid profile for all groups. Diabetic control rats showed a significant increase (*P<0.001)* in the levels of T-CH, TG, HDL-CH, and LDL-CH compared with normal rats. Yogurt supplemented with inulin and microencapsulated *L. acidophilus* was the most promising in improving plasma T-CH, and LDL-CH profile of diabetic rats. Yogurt

supplemented with inulin and microencapsulated *L. plantarum* was the most promising in improving plasma TRG and HDL-CH profile.

Table 4. Effect of different synbiotic yogurt on plasma lipid profile (mg/dL) of the studied group

Groups	T-CH (mg/dL)	TG (mg/dL)	HDL-CH (mg/dL)	*LDL-CH (mg/dL)
Control normal	77 ±2 [b]	86 ±0.2 [b]	35 ±0.7 [b]	24.8 ±2 [b]
Control diabetes	118 ±4 [a]	115 ±4 [a]	35 ±0.7 [b]	60 ±3 [a]
Group (1)	72 ±5 [b]	76 ±4 [b]	47 ±3 [a]	9.8 ±1[d]
Group (2)	74 ±2 [b]	60 ±2 [c]	48 ±2 [a]	14 ±2 [cd]
Group (3)	75 ±2 [b]	64 ±2 [c]	44 ±2 [a]	18.2 ±1 [c]
Group (4)	77 ±3 [b]	82 ±3 [b]	44 ±2 [a]	16.6 ±3 [c]
Group (5)	78 ±4 [b]	86 ±7 [b]	35 ±0.6 [b]	25.8 ±3 [b]

Each value represents the mean ± SE.

Means in the same column, followed by the same letter are not significantly different at *P<0.05*.

Friedewald Equation for Low Density Lipoprotein (LDL-CH)

*LDL-CH= T-CH – (TG/5) – HDL-CH

Ejtahed *et al.* (2012) found that administrating yogurt enriched with *B. lactis* and *L. acidophilus* has no significant effect on the levels of TG and HDL-CH in diabetic patients. Our result is consistent with the finding of Moroti *et al* (2012), who showed that administrating of a synbiotic shake supplemented with probiotics (*L. acidophilus* and *B. bifidum*) and prebiotic (oligofructose) led to increase in the level of HDL-CH. This indicates that the use of different prebiotics (inulin or oligofructose) has no influence on the therapeutic activity of probiotic (Azorín-Ortuño *et al.*, 2009). The result of the present study is in accordance with a previous study that found a positive impact for *L. plantarum* LS/07 *and L. plantarum Biocenol* LP96 on lipid profile (Salaj *et al*., 2013). Probiotic bacteria can positively affect hyperlipidemia through: (1) increasing cholesterol consumption by bacterial growth, (2) binding the cholesterol with the bacterial cell's surface, which inhibiting the cholesterol absorption by the host, (3) probiotic bacteria possessing bile acid hydrolase activity, which, as a consequence, increases cholesterol uptake and metabolism in the liver for synthetizing the bile, and (4) inhibiting the synthesis of hepatic cholesterol and triglyceride due to the presence of short-chain fatty acids such as propionic acid (Salaj *et al*., 2013; Liong and Shah, 2006; Gill and Guarner, 2004; Noh *et al*., 1997).

3.3.3. Effect of Different Synbiotic Yogurt on Liver and Kidney Function

The liver and kidney functions of all experimental groups are shown in (Table 8). Compared with the diabetic control group, the administration of synbiotic yogurt has significantly decreased (*P<0.05*) the liver enzymes (ALT and AST) and kidney enzymes (creatinine and urea) in all diabetic rat groups. The maximum decrease in ALT levels was identified in the group that was fed with yogurt supplemented with *L. rhamnosus* (26 ±1 U/L, *P=0.001*), while the maximum decrease in AST level was in rat group fed with yogurt supplemented with *B. lactis* (32 ±1 U/L, *P=0.004*). The maximum decrease in creatinine and urea levels was identified in groups fed on yogurt supplemented with *L. acidophilus* (0.72 ±0.05 mg/dL, *P=0.012*) and *L. plantarum* (30 ±0.4 mg/dL, *P=0.005*) respectively.

Table 5. Effect of different synbiotic yogurt on liver and kidney function of diabetic

Groups	ALT (U/L)	AST (U/L)	Creatinine (mg/dL)	Urea (mg/dL)
Control normal	38 ±2 [c]	32 ±0.7 [d]	0.74 ±0.04 [c]	28 ±2 [e]
Control diabetes	127 ±4 [a]	69 ±0.8 [b]	3.08 ±0.07 [a]	72 ±2 [a]
Group (1)	32 ±2 [cd]	45 ±2 [c]	0.72 ±0.05 [c]	39 ±2 [c]
Group (2)	35 ±4 [c]	34 ±1[d]	0.75 ±0.09 [c]	30 ±0.4 [de]
Group (3)	26 ±1 [d]	40 ±2 [c]	0.75 ±0.09 [c]	32 ±2 [de]
Group (4)	34 ±2 [c]	32 ±1 [d]	0.83 ±0.07 [bc]	34 ±2 [cd]
Group (5)	47 ±1 [b]	77 ±3 [a]	1 ±0.2 [b]	51 ±3 [b]

ALT (Alanine aminotransferase), AST (Aspartate aminotransferase), Each value represents the mean ± SE.

Means in the same column followed by the same letter are not significantly different at *P<0.05*.

Lucchesi *et al* (2015) observed an increase in AST and ALT after two weeks of alloxan induction, whereas ALT remained significantly elevated till 26 weeks because the liver requires longer time to cure the damage resulted from using alloxan. A previous study showed that alloxan increases the oxidative stress and reducing the oxidative defense of hepatic cells (Goel, 1977). Our result is in agreement with Bejar *et al.* (2013), who showed significant protective effects for probiotic bacteria treatment on the kidney and liver functions, which was proofed by a significant decline in serum AST, ALT, urea, and creatinine, 38.20 (U/L), 33.54 (U/L), 0.60 (g/L), and 18.78 (mg/L) respectively. Our result is in line with Kumar *et al* (2017), who showed that administration of probiotic fermented milk for 60 days has significantly decreased the urea and creatinine in animals fed with probiotics compared to the diabetic control group fed with control diet only.

Diabetes disease is associated with dysfunction and damage of liver and kidney (Ota and Ulrih, 2017). The administration of synbiotic yogurt could reduce damage to the liver and kidney by improving the metabolism of lipid and delaying the hepatic and renal disorder (Sengupta *et al*., 2019). Probiotics can also improve the liver performance by improving liver histology and decreasing the total fatty acid of the hepatic cells (Bakhshimoghaddam *et al*., 2018). The mechanism of action of probiotic bacteria on improving kidney function can be summarized as follows: (1) preventing growth of some aerobic bacteria in gut, which, in turn, enhance gut microbial balance and regulate the level of urea (Vaziri *et al*., 2013), (2) the urease activity of the probiotics can increase the degradation of the urea and, ultimately, reduce its level and enhance kidney functions (Parvez *et al*., 2006), and (3) probiotics and prebiotics can decrease the inflammatory biomarkers and the oxidative stress, which indirectly affect the performance of kidney (Grimoud *et al*., 2010).

4. Conclusion

The present work is an attempt to develop a supplementary diet incorporating the health benefits of probiotics and prebiotics. Our findings recommend the use of microcapsulation technique to maximize the benefits from probiotics. The present study suggested that synbiotic yogurt has the potential to regulate the glucose level and the lipid profile (total cholesterol, triglycerides, LDL and HDL) in diabetic rats. Furthermore, the administration of synbiotic yogurt has improved both liver and kidney functions in diabetic rats. The present study demonstrated the anti-diabetic properties of different probiotic strains.

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