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Effect of Probiotic Yogurts Enriched With Natural Sources (Fruits and Vegetables) of Inulin and FOS on Postprandial Glycemic Response in Healthy Albino Rats

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Probiotics Yogurts with natural sources of prebiotics (fruits and vegetables) are important food products that can be beneficial for diabetic individuals. This study aimed to determine the glycemic response and glycemic index of yogurts containing probiotics and natural sources of prebiotics (inulin and fructooligo saccharides). Four different levels of sweet (sweet potatoes, banana, raisins, aloe vera gel) SBRA, SBRA20%, SBRA40%, SBRA60%, and salty (tomato, garlic, onion, aloe vera gel) TGOA, TGOA20%, TGOA40%, TGOA60% probiotic yogurts were developed while plain probiotic yogurt was treated as control. A total of 81 healthy albino rats were divided into 9 equal groups and fed with these yogurts for 6 six weeks through the gavage method. On the last week of the trial glycemic response of reference foods (glucose) (on the 38th day) and tested foods (on the 42nd day) were measured. IAUC and the glycemic index of all yogurts were also calculated. The mean GI of salty yogurts TGOA40% and TGOA60% was significantly lower than control yogurt 37.94±1.44. Overall GI of all sweet and salty yogurts came under the category of low GI foods (<55). It was concluded that developed yogurt products can be used as a good substitute for diabetic patients in maintaining their blood glucose levels.

Keywords: Probiotic yogurt, inulin, fructooligo saccharides, glycemic response, glycemic index

INTRODUCTION

Diabetes mellitus come under the category of lifestyle-related disease and in the last two decades, an epidemic situation has been created due to an increased number of diabetic individuals (Wang *et al*., 2016; Collaborators, 2017). As an urgent public health priority, treating diabetes and its complications is a high social and economic burden globally (Eating, 2016; Organization, 2016). According to WHO, in the year 2030, the seventh main cause of death all over the world will be diabetes mellitus. A sedentary lifestyle along with imbalanced and processed diets are the major risk factors for diabetes mellitus type 2 (Basiak-Rasala et al.2019). Diets having a good amount of prebiotics have been proved by different epidemiological studies to be effective in the reduction of risks of these lifestyle-related diseases (Pedersen et al. 2016; Shoaib et al. 2016).

The blood glucose level elicited after intake of a meal is called glycemic response (GR) (Wolever and Jenkins, 1986). There are many ways to calculate GR but for glycemic index (GI) determination the incremental area under the glucose response curve (IAUC) over 120-150 minutes is used (Wolever, 2004). The source of carbohydrates and their quantity are responsible for GR (Wolever and Bolognesi, 1996). The formula used for the determination of GI is given below:
$GI=100\times F/G$
F and G are IAUC (glycemic response) after intake of food and glucose (which have the same amount of digestible carbohydrate present in tested food) respectively. The values of GI for different food commodities are classified as low (≤55), medium (56–69), and high (≥70) (Standardization, 2010). GI determination helps to investigate the quality of carbohydrates, dietary fibers, and added sugar in different foods (Ludwig et al.2018).

Yogurt a famous fermented dairy product along with prebiotics can reduce the risk factors of diabetes mellitus type 2 (Wolever, 2017). A recent meta-analysis revealed that each increase of 1 serving/day in yogurt intake might reduce the 17-18% risk of diabetes (Chen et al. 2014). Yogurt maintains the homeostasis of glucose in the body as it contains calcium, magnesium, conjugated linoleic acid, and vitamin D while at the same time it also enhances gut activity through increasing microbial load (Rice et al. 2011;

Gomes et al. 2015). The association between supplementation of inulin-type fructans (inulin and FOS) in yogurts and glycemic control has been observed in many epidemiological studies (Lightowler et al. 2018). Inulin-type fructan comes under one category of prebiotics (inulin, FOS, and galactooligosaccharides (GOS)), and is a linear fructan with β (2-1) bonds (Wilson and Whelan, 2017). Different randomized control trials suggest that intake of inulin and FOS can exert good effects on glycemic control (Bonsu et al.2011). It has been suggested by various studies that inulin and FOS can overcome and reduce the symptoms of different metabolic problems including diabetes mellitus (Li et al. 2021).

The two major food groups, (1) milk and milk products and (2) fruits and vegetables can be combined to develop suitable food products for diabetic individuals having low GI (Basiak-Rasala et al. 2019). So in the present study yogurt from the milk group (as probiotic) and different fruits and vegetables (as prebiotics inulin and FOS) from other groups were combined to develop sweet and salty yogurts with low GI. The present study aimed to determine the GR and GI of developed sweet, salty, and control yogurts containing probiotics and prebiotics.

MATERIALS AND METHODS

Development of different levels of yogurts:

Firstly, plain yogurt was developed by using the method of Kebary, (2020); having starter culture (*S. thermophilus* and *L. bulgaricus*) with a ratio of 1% (1% v/v). Further, sweet and salty yogurts were developed with different levels of dried fruit and vegetables; having 150ml probiotic yogurt in each serving mentioned in table 1.The strain of *L. acidophilus* was used as probiotics with at least 10^9 colony forming units (CFU) per portion of yogurt.

Preparation of experimental settings and doses:

The total study duration was of 6 weeks in which a total of 81 healthy albino rats having an average body weight of 150gm, were selected and divided into 9 equal groups. There were two trials of sweet and salty yogurts having equal four groups of rats while one group was selected as a plain yogurt control group. All rat groups received two doses (one in the evening and one in the morning) of respective sweet, salty, and control yogurts daily according to their body weights by using the formula described by Erhirhie et al.(2014). The total amount of digestible carbohydrates in each type of yogurt was determined by using proximate analysis (AOAC methods) (Chemists and Horwitz, 2000). GR of sweet, salty, and control yogurts was determined by measuring blood glucose level on the last day (42^{nd} day) of both trials (sweet and salty). Glucose/sugars dissolved in water present in one serving of sweet, salty, and control yogurts were used as standard foods and fed to rats to their respective groups on 38^{th} day of trial. The gap of days in analysis was used to recover the rats from stress caused by handling, pricking on tail and fasting conditions.

A standard basal diet that was given to rats was *Iso-caloric and iso-nitrogenous*; containing all essential and required nutrients and provided to all experimental rats ad libitum (Reeves, 1993). The experimental diets and standard foods were given through the gavage method described by (Chen et al. 2019).Experimental diets were ground, and mixed with distilled water while in standard food, glucose was simply dissolved in distilled water and then given to rats (Presented in table 2).All rats were normal in health and were not affected by diabetes. To rule out random and fasting diabetes in rats, a glucose tolerance test was conducted before the study (Association, 2015). Analysis of postprandial blood glucose response was conducted after 12 hours of fasting.

Blood samples were taken through a prick capillary lancing device and blood glucose concentrations were measured through a blood-glucose meter (Joint & Organization, 1998; Wolever et al. 2008). Tail was used to take blood samples. The needle pricking method was used to take blood samples by using a disposable and sterile lancing device. Blood glucose level was determined firstly at fasting then after 30, 45, 60, 90, and 150 minutes of taking one serving of developed sweet, salty, control yogurts and standard foods (Brites et al. 2011). Serum glucose levels were determined using a glucometer withCerteza medical® (Changsha Sinocare Inc. China) blood glucose test strips. Blood glucose measurements were performed by a qualified laboratory technician. The study protocol for GR and GI was carried out by using the method and guidelines of (Brouns et al. 2005) and FAO/WHO (Hujoel, 2009). The incremental area under the curve (IAUC) was total blood glucose response and it was calculated for all sweet, salty, and control yogurt as well as for their respective standard foods according to the method described by FAO/WHO (Hujoel, 2009; Wolever et al.1991). The mean values for all IAUC were used to find out the GI for each type of yogurt. The following formula was used to calculate GI values.

GI= (IAUC tested experimental food/IAUC standard food)×100 (Wolever et al. 1991)

Statistical analysis:

Statistical Software Minitab 17 was used for the statistical representation of data. Nine samples in each group were used for mean, standard deviations, and standard error mean (SEM) to present all statistical data. Two-way analysis of variance (ANOVA) was used for analysis in which different levels of yogurt treatments and two varieties of yogurts (sweet and salty) were used as factors. Furthermore, for the comparison of means Tukey's test was used.

Table 1: Weight of fresh and dried fruits and vegetables (grams) added to sweet and salty probiotic yogurts (150ml) based on pre-determined Inulin and FOS content

Sweet yogurts					
Fruit/Vegetable (g)	SBRA		SBRA20%	BRA40%	SBRA60%
	FW	DW	DW	DW	DW
Sweet potato	80	6.24	33.48	39.06	44.64
Banana	20	6.92	38.10	44.94	51.36
Raisins	40	7.72	44.51	51.93	59.34
Aloe vera gel	40	1.07	1.28	1.50	1.71
Salty yogurts					
Vegetable (g)	TGOA		TGOA20%	TGOA40%	TGOA60%
	FW	DW	DW	DW	DW
Tomato	80	6.24	7.49	8.74	9.98
Garlic	20	6.92	8.30	9.69	11.07
Onion	40	7.72	9.26	10.81	12.35
Aloe vera gel	40	1.07	1.28	1.50	1.71

FW=fresh weight; DW=dry weight SBRA=Sweet potato, Banana, Raisins, Aloe vera gel TGOA= Tomato, Garlic, Onion, Aloe vera gel

Table 2: Amount of one dose of sweet, salty, and control yogurts along with their respective amount of digestible carbohydrates (g) fed to rats through the gavage method

Sweet yogurt groups	**SBRA**	**SBRA20%**	**SBRA40%**	**SBRA60%**
One dose (g)	0.63	0.68	0.73	0.78
Digestible carbohydrates (g)	0.23	0.27	0.30	0.33
Salty yogurt groups	**TGOA**	**TGOA20%**	**TGOA40%**	**TGOA60%**
One dose (g)	0.44	0.45	0.46	0.48
Digestible carbohydrates (g)	0.06	0.07	0.07	0.08
Control	**Plain yogurt**			
One dose (g)	0.30			
Digestible carbohydrates (g)	0.02			

SBRA=sweet potato, banana, raisins, aloe vera gel; TGOA= tomato, garlic, onion, aloe vera gel

RESULTS

Determination of glycemic response

The postprandial blood glucose response curve of the sweet, salty, and control yogurts in the first and second trials showed different profiles which have been presented in Fig 1. The rats undergoing sweet, salty, and control treatments showed peak blood glucose concentration after 30 minutes of consumption. The highest blood glucose concentration was exhibited by sweet yogurt TGOA60% (127.78±6.42). The lowest value of peak postprandial blood glucose concentration was exhibited by rats who consumed salty yogurt TGOA (93.67±2.40 mg/dl) while the control group showed (97.89±3.55 mg/dl) peak postprandial blood glucose concentration. There was a significant difference in peak postprandial blood glucose concentration values among control and all yogurt treatments as well as a significant difference was also observed between sweet and salty yogurts. The post-prandial blood glucose concentrations were lower in salty yogurts when compared with sweet yogurts.

The mean IAUC and GI of all sweet, salty, and control yogurt treatments have been shown in table 3. The mean IAUC was significantly different in control and all yogurt treatments (P<0.05). There was also a significant difference in IAUC between both sweet and salty yogurts (P<0.05).

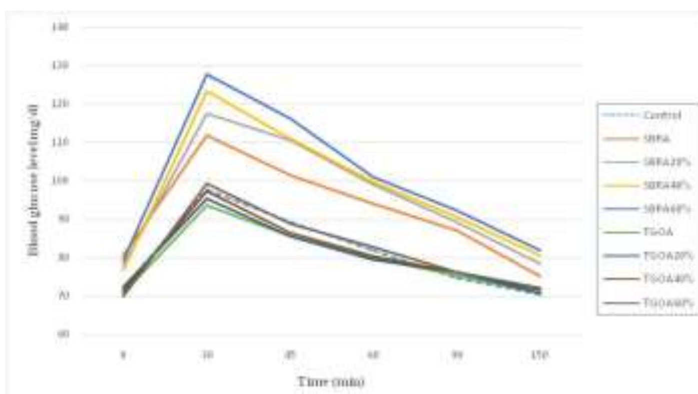

Figure 1: Average postprandial blood glucose response of the rats after consuming sweet, salty, and control yogurts

IAUC was lower in control yogurt than sweet yogurts (SBRA, SBRA20%, SBRA40%, and SBRA60%) and salty yogurts (TGOA40% and TGOA60%) and was higher than salty yogurts TGOA and TGOA20%. Maximum IAUC was shown in sweet yogurt SBRA60% (14697) while it was lowest in the salty yogurt TGOA (11773). Reduced IAUC of salty yogurts than sweet yogurts showed the effectiveness of salty yogurts in reducing post-prandial blood glucose levels. Results revealed that salty yogurts might show their effectiveness in controlling diabetes mellitus.

Table 3: Results of peak postprandial blood glucose concentration levels, incremental area under the curve (IAUC), and GI values

Groups	Peak serum glucose concentrations (mg/dL)	IAUC ((mg/dL) min)	Glycemic Index (GI)
Control	97.89±3.55[de]	11944±136[d]	37.94±1.44[cd]
Sweet yogurts			
SBRA	112.00±3.16[c]	13546±299[c]	40.16±2.90[abc]
SBRA20%	117.67±2.40[b]	14077±100[b]	41.61±2.98[ab]
SBRA40%	123.44±5.68[a]	14334±265[b]	42.11±3.24[a]
SBRA60%	127.78±6.42[a]	14697±341[a]	42.19±2.91[a]
Salty yogurts			
TGOA	93.67±2.40[e]	11773±192[d]	39.57±2.65[abc]
TGOA20%	95.33±2.69[de]	11858±132[d]	38.33±1.97[bcd]
TGOA40%	97.44±1.67[de]	11956±110[d]	37.28±1.36[cd]
TGOA60%	99.33±1.32[d]	12073±218[d]	35.85±1.40[d]
SEM	13.20	43748	5.512
Two-way ANOVA			
Treatments levels	0.00	0.00	0.063
Yogurts variety	0.00	0.00	0.00
Interactions	0.00	0.00	0.00
The mean is a replicate of nine values			

SBRA=sweet potato, banana, raisins, aloe vera gel; TGOA= tomato, garlic, onion, aloe vera gel

The mean GI of sweet and salty yogurts ranged from (35.85-42.19) which was showing that all experimental and control yogurts had a low GI category (1-55). There was a non-significant difference in all yogurt treatments (P>0.05) but a significant difference was observed between sweet and salty yogurts (P<0.05). In sweet and salty yogurts highest and lowest GI were 42.19 (SBRA60%), 40.16 (SBRA), and 39.57 (TGOA), 35.85 (TGOA60%), respectively. Overall the highest and lowest GI were 42.19 and 35.85 observed in sweet SBRA60% and salty TGOA yogurts respectively. The control yogurt had a glycemic index value of 37.94.

DISCUSSION

Dairy products are considered low GI food (<55) and yogurt is one of the most important dairy food. 95% of dairy foods fall in the low GI foods category (Atkinson et al. 2021). In the year 2008 a study conducted to find out international tables of GI for different food commodities showed that fruit yogurt might have a GI value of 41±2 (Atkinson et al.2008). Prebiotic fibers especially inulin and FOS can control the adverse effects of diabetes and also exert some potential in its management and prevention (Anderson et al. 2009; Gargari et al. 2013). Therefore in this research different sweet and salty yogurts were developed having fruit and vegetables as sources of prebiotics (inulin and FOS) to check their GI to find out their effectiveness for diabetes mellitus and other health issues. In the present study GR of all yogurts showed that Peak serum glucose concentration was not more than 127.78±6.42 (mg/dL) in sweet yogurt SBRA60% while the lowest was 93.67±2.40 in salty yogurt TGOA. These low ranged values were showing low GR of yogurts. This reduced GR in all yogurts was due to the production of lactic acid during the fermentation process in yogurts. And the presence of prebiotics in the form of added fruits and vegetables in sweet and salty yogurts might enhanced this activity. This lactic acid has been previously proved to reduce the GR by delayed stomach emptying (Liljeberg and Björck, 1998).

In the present study IAUC was highest in sweet yogurt SBRA60% (14697±341) and lowest in salty yogurt TGOA (11773±192) while in plain or control yogurt it was 11944±136. Although both sweet and salty yogurts contained inulin and FOS in the form of added fruits and vegetables in yogurts this wide range of iAUC was due to more sugar content in sweet yogurts (due to the presence of raisins and banana) than in salty yogurts. In a previous study iAUC in plain, cinnamon, and turmeric added novel yogurts were11951±523, 11012±611, and 10941±530 respectively ((mg/dL)min) (Pavalakumar et al. 2020). These values were very close and within the range of the present study. Increased IAUC in sweet yogurts and reduced in salty yogurts was showing that GI of sweet yogurts will be higher than salty yogurts but all yogurts exhibited low ranged GI (<55). Another study conducted on goat milk yogurt fortified with raisins revealed that the GI and IAUC of this yogurt were 47.44±9.44 and 946.08±242.56 respectively. These values of GI were much closed to sweet yogurts of our present study but IAUC was very much lesser than present study which was maybe due to the difference between animal and human study (Papakonstantinou et al. 2022).

In the present study the GI of all sweet and salty yogurts was higher than the control group only except for TGOA40% AND TGOA60%. But the values of GI of all yogurts were within the range of the lower GI food category (<55). The amount of carbohydrates was controlled in developed yogurts which were tested for GI. Fibers, protein, and fat contents were the main reasons to elicit GR and then further GI. In the present study sweet and salty yogurts contained a high amount of inulin and FOS than control yogurt but at the same time sweet yogurts contained more amount of digestible carbohydrates than control and salty yogurts which was the main reason for high GI of sweet yogurts than control yogurt but still, it was falling under the low GI food category. In a review, it was evaluated that the mean GI of plain and sweet yogurts (added fruits and sugars) were 27 and 41 respectively. In the present study values of GI of sweet yogurts were within the range of this value but the value of control yogurt was higher than plain yogurt but still came under the low category (Wolever, 2017). To check the effect of inulin supplementation on humans a study was conducted in which 10 g/day of inulin or maltodextrin (control) were given to diabetic patients for 2 months. Inulin supplementation significantly reduced the HbA1c as well as fasting plasma glucose levels (Gargari et al. 2013). Intake of 10 g/day inulin for 8 weeks in healthy subjects (Jackson et al.1999) and a similar dose of FOS for 2 months in individuals with mild hypercholesterolemia in another study showed good results for fasting serum glucose levels (Giacco et al. 2004). Similarly reduction in HbA1c levels was observed in healthy young adults when inulin-enriched pasta was given to them (Russo et al. 2010).

All these developed yogurts may be proved to be effective products for diabetes. Sweet yogurts showed a higher GI than control and salty yogurts due to the presence of banana and raisins in them which also contained a good amount of disaccharides. Salty yogurts showed lower glycemic index values due to the presence of more fiber content and lesser sugars in them. The hypoglycemic effect of inulin and FOS has been explained by several mechanisms. Postprandial blood glucose concentration can be reduced by delayed gastric emptying which retard the entrance of glucose into the blood (Jovanovski et al. 2019). The presence of prebiotics in diet also modifies the secretions of some gut hormones (GLP-1) (Cani and Delzenne, 2009) as well as in the colon the production of SCFA by the fermentation of these prebiotics plays a crucial role in maintaining blood glucose and insulin levels. The production of SCFA also inhibits gastric emptying when nutrients reached the ileocolonic junction (Cherbut, 2003). Intake of FOS increases the levels of pancreatic insulin and β-cell, plasma insulin, GLP-1, and GLP-2 (Cani et al. 2005; Cani et al. 2006). In the present study, sweet and salty yogurts contained probiotics *L. acidophiulus* and *B.bifidum* which also proved to be effective in showing GR within the range as well as GI of all yogurts in the low category. As a previous study showed that multi-strain probiotics exerted good effects in diabetic individuals (type 2 diabetes) by showing improvements in fasting insulin and HbA1c (Firouzi et al. 2017).

CONCLUSION

In the present study, it was concluded that probiotic yogurts are a good source of prebiotics (inulin and FOS). All yogurts showed a glycemic response in a range not greater than127.78±6.42 mg/dl (peak blood glucose concentration). GI of all yogurts came under the low GI food category (<55) but salty yogurts TGOA40% and TGOA60% showed the lowest GI values 37.28±1.36 and 35.85±1.40 respectively which made them the best option for diabetic patients

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

RS gave the main idea and conceptualization, did laboratory investigation, and also wrote the manuscript. AH supervised and evaluated the research. MUN and ZUNH supervised too, evaluated and provided laboratory facilities. All authors gave their final satisfactory verdict to manuscript.

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