CHAPTER-4

Prebiotic activity of enzymatically modified pea peel dietary fiber: an *in vitro* study

4.1. Introduction

It is widely recognized that populations consuming diets high in dietary fiber (DF) have a lower incidence of chronic diseases compared to populations which consume diets lower in DF [2, 11]. Weak immune system, increased inflammation, and other health issues are caused by low and unbalanced levels of probiotics, which are helpful gut microorganisms. A high-fiber diet can increase the number of beneficial bacteria in the gut. Dietary fiber is a emerging source of prebiotics, is resistant to digestion by enzymes, and functions as a particular carbon source to promote the activity of gut microbial cells. [16]. Prebiotics boost immune response by modulating gut microbial activity and production of SCFA (short chain fatty acids) [15]. Prebiotics have substantial health advantages because they can selectively encourage the development of bifidobacteria and lactobacilli in the distal parts of the colon. The oligosaccharides are the substances that matter the most. Among other impacts, these prebiotics may have additional bioactive qualities that improve mineral absorption, treat metabolic problems, and reduce stomach emptying. [4]. The ability of oligosaccharides to resist digestion in the upper gastrointestinal system is one need for them to be regarded as prebiotics. The chemical structure has a significant impact on the prebiotic oligosaccharide's hydrolysis susceptibility during transit through the GI tract, which can have an effect on the oligosaccharide's ultimate state when it reaches the colon to be fermented by the microbiota. The bacteria that are most frequently employed as probiotics are from the species Bifidobacterium and the diverse group of lactic acid bacteria (*Lactobacillus, Enterococcus*). Widespread use of these bacteria has been made in both dairy and non-dairy products [5]. Probiotics are frequently ingested as nutritional supplements or as a component of fermented foods with specially added active living cultures, such as yoghurt and soy yoghurt. Probiotics have been shown to be helpful for persons with gastrointestinal illnesses, including food allergies, inflammatory bowel diseases, celiac disease, and infectious diarrhoea, among many others [17]. Enzymatic techniques are used to change the solubility of dietary fiber in order to increase its health advantages

and greater purity [10]. The capacity of dietary fiber to enhance the development of probiotics has recently been described in a research [14; 2]. However, the current research on modified dietary fiber suggests that it may have prebiotic action due to its high water solubility. Prebiotics are nondigestible food ingredients; for example, inulin, xylooligosaccharides (XOS), and fructooligosaccharides (FOS). These fibers generally provide several advantages to consumers by selectively stimulating the growth of useful colonic bacteria within a human's colon [14]. *Lactobacillus* spp. are a major part of the lactic acid bacteria group, because most of its members convert lactose and other sugars to lactic acid. The production of lactic acid inhibits the growth of some harmful bacteria [17].

To serve the increase of functional fiber demand, quantity and variety of prebiotic fibers are needed. Modification of dietary fiber from pea peel using an enzymatic approach might provide a new prebiotic fiber. To our knowledge, limited studies have been carried out on the digestibility of pea peel insoluble dietary fiber. Establishing the digestibility of prebiotic carbohydrates is of great practical application, since this influence the final dose of substrate that reaches the distal portions of gut to exert its prebiotic effect. Thus, the aim of this work has been to investigate the ability of dietary fiber to serve as prebiotics and as carbon sources for probiotics and the changes in the dietary fiber fraction using standardised *in vitro* digestive conditions with a more physiological relevant gastric digestion approach.

4.2. Materials and methods

4.2.1. Materials and bacterial strains

Green peas were procured from growers of Tezpur, Assam and green pea peels (GPP) were separated manually and dried in a tray dryer (Model # BDI-51, Labotech, Make # Delhi, India). Cellulase (300 U/g), xylanase (2500 U/g), and all other chemicals were of high purity analytical grade (Sigma-Aldrich Co).

Lactobacillus rhamnosus ATCC 7469, *Lactobacillus sakei* ATCC 15521, *Lactobacillus plantarum* ATCC 8014; these lactic acid bacteria represented as probiotic strains. *Escherichia coli* ATCC 4157; was used as a pathogenic bacterium. All bacteria were maintained in glycerol stock at − 20°C. All of the microbial strains were obtained from HiMedia, India.

4.2.2. Extraction and enzymatic modification of pea peel insoluble dietary fiber

The insoluble dietary fiber (IDF) was extracted from pea peel using ultrasound-assisted alkaline extraction with NaOH 1.2%, extraction time 30 min, solid to liquid ratio 1: 30, and ultrasonic amplitude 30% under controlled conditions using a probe-type ultrasonicator (Model: Q700-200 Digital Sonicator, Qsonica LLC, Make: India). The extraction and modification study has been already done by the team [10]. Furthermore, the IDF was modified using enzymes (cellulase 90U/g and xylanase 21U/g) concentrations and incubated at 50°C for 120 min [10]. After enzymolysis, 4 times 95% ethanol was added to the solution followed by cooling (25±1°C) and drying in hot air oven (50°C).

4.2.3. Prebiotic activity

4.2.3.1. Inoculum preparation

L. rhamnosus, *L. sakei*, and *L. plantarum* were cultured in MRS broth at 37°C for 24 h. One loop of cultured media was transferred to 10 ml of fresh MRS broth and incubated at 37°C for 12 h. Subsequently, the strains (active bacterial cells) were centrifuged at 10,732× g relative centrifugal force (RCF) for 10 min. The biomass of each strain was washed twice with 0.85% (w/v) normal saline. Afterward, the optical density (OD) at 600 nm of each cell solution was adjusted to 0.5 (10^8 CFU/ml). *Escherichia coli* were cultured under the same conditions as mentioned above in a nutrient broth.

4.2.3.2. The consumption of dietary fiber as a sole carbon source by prebiotics

To assess with certainty the effect of pea peel dietary fiber on probiotic growth, a glucose medium was taken as the control. The concentration of glucose in the control medium relied on the exact glucose content in the dietary fiber solution. First, the concentration of glucose in the dietary fiber solution was determined by the Nelson-Somogyi method or dinitrosalcylic (DNS) method. Second, the glucose control was prepared by adding glucose to glucose-free MRS to obtain control (Table 4.1) The glucose free dietary fiber (GFDF), media with glucose in MRS as (+G), and glucose-free MRS (-G) media, media with inulin (+I) were inoculated with probiotic strains and subsequently incubated at 37°C for 48 h. The samples were collected at 0, 24, and 48 h to measure, the glucose remaining, and the optical density of cells [14]. These experiments aimed to investigate

the possibility of the bacteria to use dietary fibers as a sole carbon in the low-glucose-containing broth, thus; the number of bacterial cells was expressed as the cells accumulation instead of colony forming unit.

Table 4.1 MRS media composition (1L) (Kang et al., 2022)

Reagents	Quantity in 1L
Peptone	10 g
Meat extract	8 g
Yeast extract	4 g
Dipotassium hydrogen phosphate	2 g
Sodium acetate trihydrate	5 g
Triammonium citrate	2 g
Magnesium sulphate heptahyrate	0.2 g
Manganous sulphate tetrahydrate	0.05g
Glucose	20 g
Tween 80	10 mL

4.2.3.3. Prebiotic activity test

Prebiotic activity was determined using given formula (Eq 2). Probiotics and pathogenic bacteria were used in this experiment; as a result, lactic acid bacteria were cultivated in MRS and the pathogen was cultured in nutritional broth (NB).Media consisting of 1 g/L of dietary fiber were prepared (+DF). The GF medium (-G), 1 g/L inulin medium (+I), and 1 g/L glucose medium (+G) were used as control media. The fermentation condition was 37°C for 48 h. The samples were collected at 0, 24, and 48 h of cultivation to measure cell growth by OD_{600}. The relative growth (RG) and prebiotic activity index (PI) [14] were determined using the following equations:

$$\text{Relative Growth (RG)} = \frac{(P^{24}_{p} - P^{0}_{p}) - (P^{24}_{NG} - P^{0}_{NG})}{(P^{24}_{G} - P^{0}_{G}) - (P^{24}_{NG} - P^{0}_{NG})} \quad (1)$$

$$\text{Prebiotic Index (PI)} = \left[\frac{(P^{24}_{p} - P^{0}_{p}) - (P^{24}_{GF} - P^{0}_{GF})}{(P^{24}_{G} - P^{0}_{G}) - (P^{24}_{GF} - P^{0}_{GF})}\right] - \left[\frac{(E^{24}_{p} - E^{0}_{p}) - (E^{24}_{GF} - E^{0}_{GF})}{(E^{24}_{G} - E^{0}_{G}) - (E^{24}_{GF} - E^{0}_{GF})}\right] \quad (2)$$

Where,

P^{0}_{p}, P^{24}_{p} are OD_{600} values for probiotics at 0 and 24 h in prebiotic containing media (+DF)

P^{0}_{GF}, P^{24}_{GF} are OD_{600} values for probiotics at 0 and 24 h in glucose-free media (-G)

P^0_G, P^{24}_G are OD_{600} values for probiotics at 0 and 24 h in glucose containing media (+G)
P^0_{NG}, P^{24}_{NG} are OD_{600} values for probiotics at 0 and 24 h in inulin containing media (+I)

E^0_p, E^{24}_p are OD_{600} values for pathogen at 0 and 24 h in prebiotic containing media (+DF)
E^0_{GF}, E^{24}_p are OD_{600} values for pathogen at 0 and 24 h in glucose-free media (-G)
E^0_G, E^{24}_G are OD_{600} values for pathogen at 0 and 24 h in glucose containing media (+G)

4.2.4. *In vitro* gastrointestinal digestion

The solutions used for the simulation of the oral and gastric phases were based on the standardised static digestion protocol [4]. 5 mL of sample was placed into a 70 mL glass v-form vessel thermostated at 37°C. To simulate the oral phase, 4 mL of Simulated Salivary Fluid (SSF), **Table 4.2,** [6], 25 mL 0.3 M $CaCl_2(H_2O)_2$ and 0.975 mL Milli-Q water were added and mixed for approximately 2 min using a shaker at 35 rpm. The simulation of the gastric phase was conducted using a semi-dynamic model described by Ozorio et al. [13]. The gastric fluids and enzyme solution were added gradually. Two solutions were added at a constant rate for 2 h: (1) 9 mL of a mixture consisted of 88.9% Simulated Gastric Fluid (SGF), Table S5 0.06% 0.3 M $CaCl_2(H_2O)_2$, 4.4% Milli-Q water and 6.7% 2 M HCl was added; and (2) 1 mL of pepsin (3214 U/mg solid, using haemoglobin as substrate) solution (in water) was added to reach the protease activity of 2000 U/mL in the final digestion mixture. This enzyme solution was added using a syringe and system was agitated using the shaker at 35 rpm during the digestion time. The pH was recorded throughout the procedure.

Samples (0.5 mL) were taken after 0, 1 and 2 h of digestion and the pepsin activity was stopped with 100 µL of 1 M $NaHCO_3$ for a subsequent analysis of the protein fraction and the rest of the sample with 150 µL of 5 M NaOH for the following intestinal digestion. This last sample was labelled as G-Phase (**Table 4.4**) sample. After gastric digestion, small intestinal digestion was carried out; The rest of the liquid G-Phase (~16.5 mL) was subjected to the small intestine conditions following the protocol [4]. The digestion was carried out at 37°C for 2 h. Samples (5 mL) were taken at 0, 1 and 2 h of small intestinal digestion, which were respectively labelled as 0-IPhase, 1-IPhase and 2-IPhase. They were freeze-dried until further analysis. Dietary fiber and glucose estimation was performed for all the samples kept in the freeze drier.

Table 4.2 Composition of simulated salivary fluid (SSF) at pH- 7.4 (Guhmann et al., 2012)

Reagents	SSF
Potassium dihydrogen phosphate (KH_2PO_4)	12 mM
Sodium chloride (NaCl)	40 mM
Calcium Chloride ($CaCl_2$)	1.5 mM
Sodium hydroxide (NaOH)	To pH 7.4
Demineralized water	To 1L
α-amylase	at 150 units/mL of SSF

Table 4.3 Composition of simulated intestinal fluid (SIF) at pH- 6.8 (Ozorio et al., 2020)

Reagents	SIF (mM)
Potassium chloride (KCl)	6.8
Monopotassium phosphate (KH_2PO_3)	0.8
Sodium hydrogen carbonate ($NaHCO_3$)	85
Sodium chloride (NaCl)	38.4
Magnesium dichloride ($MgCL_2(H2O)_6$)	0.33
Calcium chloride ($CaCl_2(H_2O)_2$)	0.6
HCl	8.4

Table 4.4 Composition of simulated gastric fluid (SGF) at pH- 2.1 (Ozorio et al., 2020)

Reagents	SGF (mM)
Potassium chloride (KCl)	6.9
Monopotassium phosphate (KH_2PO_3)	0.9
Sodium hydrogen carbonate ($NaHCO_3$)	25
Sodium chloride (NaCl)	47.2
Magnesium dichloride ($MgCL_2(H_2O)_6$)	0.12
Calcium chloride ($CaCl_2(H_2O)_2$)	0.15
HCl	15.6
Ammonium carbonate $(NH_4)_2CO_3$	0.5

4.2.5. Statistical analysis

Graphs were created using the Origin 8.5 software (Origin Lab Corporation, Northampton, USA). Using SPSS Statistics 17.0 (IBM, Chicago, USA) software, Duncan's test was performed on the data from the triplicate experiment. Separate letters in superscript were used to indicate the significant difference at $p \leq 0.05$.

4.3. Results and discussion

4.3.1 The consumption of dietary fiber as carbon sources by prebiotics

The utilization of pea peel dietary fiber as carbon source reveals that MDF provide an excellent support for relative growth of the probiotic micro-organisms. The results showed that the media containing MDF could stimulate the relative growth of the all

probiotics; *LR*, *LS*, and *LP*, higher than that of media containing UDF seen in Table 4.1. The relative growth of the bacteria, as recorded by optical density for media containing MDF for *LB*, *LS* and *LP* were recorded as 0.74, 0.856 and 0.748 respectively which were comparatively higher than pathogenic bacteria *E. coli* which was 0.26. Whereas, the media containing UDF, the cell density as OD significantly drop for all probiotic bacteria which ranges from 0.029 to 0.066 but supported the growth of *E.coli* which was 0.271. The plausible reason could be due to MDF has less impurities after enzymatic modification treatment and thus has more BET surface area [10] comparing to UDF which may allow the probiotic bacteria rapidly to utilize the fiber. However, with increase in the time, the quantity of DF decreased as it was utilized more as carbon source by microbes which limit its quantity in media. Thus, later micro-organisms could not able to grow continuously and started to lyse themselves. This could be the reason for the slight reduction in the turbidity of the medium that was incubated over 24 h. Consequently, it can be concluded that for *LB*, *LS* and *LP* are able to perfectly utilize MDF more as a carbon source than UDF. This result relates to the findings of Phirom-on et al. [14] where cellulose-based banana peel fiber was significantly utilized by probiotics *L. plantarum* SKKL1 and *L. casei* TISTR1463. In conclusion, the degree of sugar polymerization is one of the crucial factors affecting the availability of carbohydrates for consumption by bacteria. Thus MDF could be the potential prebiotic fiber which can provide sugars which was highly utilized by probiotic bacterias.

4.3.2 Prebiotic activity

Prebiotic index is the quantitative score that describes the prebiotic effect. It measure the comparison of different prebiotic carbohydrate, indicates that the growth of microorganism is stimulated by prebiotic in comparison to the control carbohydrate that is pathogenic microbial growth thus, it was calculated by using them as a substitute of carbon source in replacement of glucose [15, 9]. The result shows that prebiotic activity was highly supported by MDF than UDF for all probiotic bacterial strains which had PI; 0.534, 0.607 and 0.544 for *LR*, *LS* and *LP* respectively except for the pathogenic bacteria i.e, *E. coli* which had 0.008 as shown in **Table 4.6**. *L. sakei* achieved the maximum PI of 0.607 where microbial growth was calculated as optical density value at 600 nm followed by *L. plantarum*, and *L. rhamnosus* in MDF medium. As opposed to the MDF medium, the PI in the UDF medium was 0.141, 0.084, and 0.145 for the *LR*, *LS*, and *LP*,

respectively. The MDF medium showed that all probiotic microorganisms could readily grow, since glucose is a common sugar for living cells which can obtained by modification of DF. Commercial prebiotics, such as inulin, can selectively promote the growth of probiotic strains [14]. It was also found that in the presence of MDF, *Escherichia coli* did not grow well as its prebiotic index value was 0.008 (**Table 4.6**). Since probiotic bacteria grew very well in MDF but pathogenic bacteria did not. This result supported by Phirom-On et al. [14] where probiotics were able to produce β-1, 2-glycosidase, which is the inulin degradation enzyme, much better than pathogenic bacteria could [14]. Diaz-Vela et al. [3] reported positive prebiotic activity values for *L. rhamnosus* GG with pineapple peel flour and cactus pear flour (0.19 and 0.21, respectively). Thus, MDF from pea peel showed positive prebiotic property than UDF due to its availability of simple sugars utilized by probiotic microorganisms.

Table 4.5 *Lactobacillus rhamnosus* ATCC 7469, *Lactobacillus sakei* ATCC 15521, *Lactobacillus plantarum* ATCC 8014 *and Escherichia coli* ATCC 4157 growth at 0, 24 and 48h measured with OD at 600nm

Lactobacillus rhamnosus growth	0 h	24 h	48 h
+G	0.390±0.026	2.139±0.006	-
-G	0.322±0.085	0.363±0.008	1.159±0.143
+I	0.038±0.013	0.528±0.005	0.884±0.044
+ MDF	0.458±0.028	1.887±0.295	-
+ UDF	0.105±0.054	0.674±0.007	-
Lactobacillus sakei growth			
+G	0.06±0.047	0.409±0.047	-
-G	0.053±0.042	0.141±0.017	0.832±0.045
+I	0.082±0.014	0.222±0.023	0.789±0.046
+ MDF	0.265±0.115	0.584±0.03	-
+ UDF	0.253±0.089	0.407±0.036	-
Lactobacillus plantarum growth			
+G	0.392±0.023	2.123±0.004	-
-G	0.321±0.091	0.355±0.007	1.156±0.145
+I	0.037±0.012	0.567±0.005	0.963±0.034
+ MDF	0.491±0.022	1.92±0.293	-
+ UDF	0.110±0.055	0.676±0.006	-
E. coli growth			
+G	0.338±0.023	2.192±0.021	-
-G	0.026±0.012	0.148±0.011	0.775±0.042
+I	0.124±0.022	0.285±0.02	0.698±0.041
+ MDF	0.286±0.024	0.89±0.031	-
+ UDF	0.194± 0.023	0.815±0.03	-

Table 4.6 Relative growth at OD at 600 nm and Prebiotic Index for *Lactobacillus rhamnosus* ATCC 7469, *Lactobacillus sakei* ATCC 15521, *Lactobacillus plantarum* ATCC 8014 *and Escherichia coli* ATCC 4157

Cultures	Relative Growth (OD at 600 nm)		Prebiotic Index	
	UDF	MDF	UDF	MDF
Lactobacillus rhamnosus	0.062± 0.014^{b}	0.745±0.007^{b}	0.141±0.011^{c}	0.534±0.005^{b}
Lactobacillus sakei	0.066± 0.015^{b}	0.856±0.005^{c}	0.084±0.012^{b}	0.607±0.005^{c}
Lactobacillus plantarum	0.029±0.001^{a}	0.748±0.006^{b}	0.145±0.01^{c}	0.544±0.004^{b}
E. coli	0.271±0.024^{c}	0.26±0.022^{a}	0.01±0.001^{a}	0.008±0.001^{a}

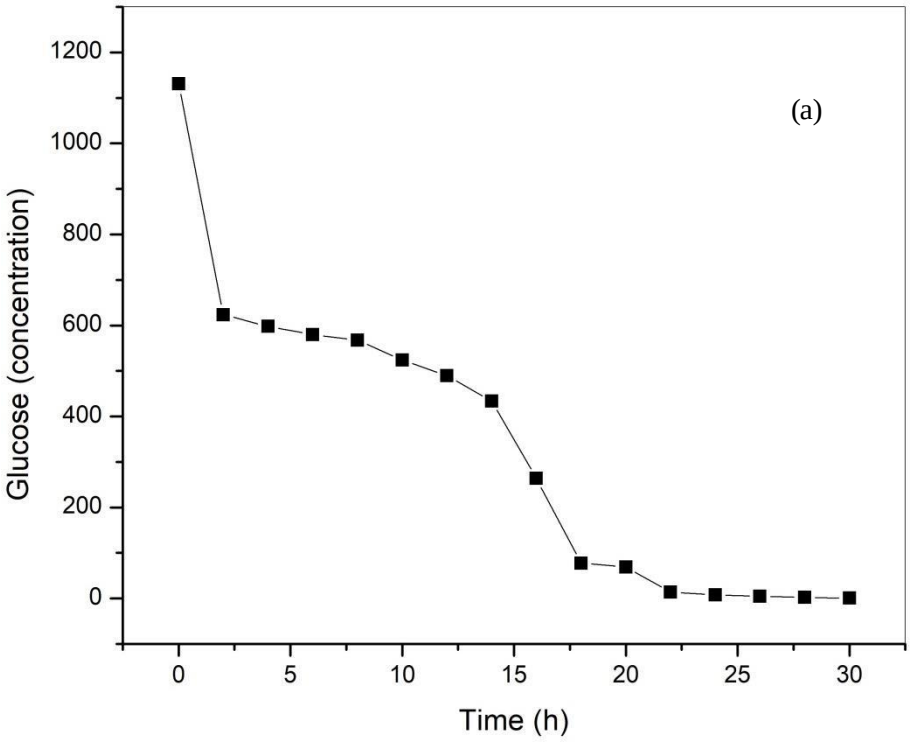
UDF: unmodified insoluble dietary fiber; MDF: modified insoluble dietary fiber

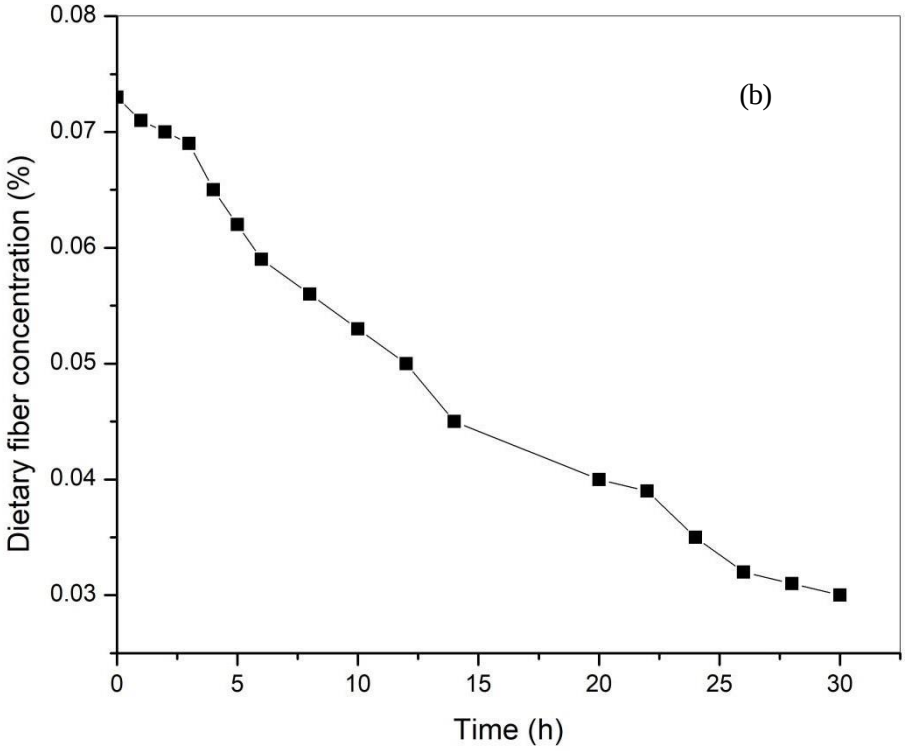
4.3.3 Growth kinetics of the *Lactobacillus* strain in glucose and dietary fiber medium

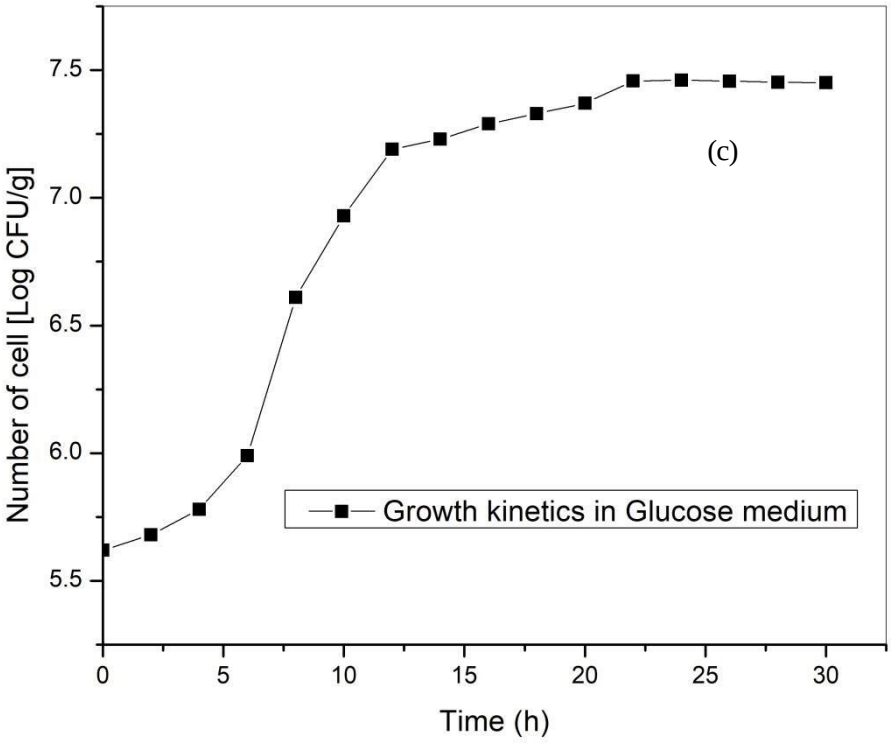
After 30 h of incubation, cell density obtained from *Lactobacillus rhamnosus* ATCC 7469 culture conducted in MRS was 7.45 ± 0.08 log CFU/mL in glucose medium and 7.37 ± 0.07 log (CFU/mL) in dietary fiber medium. The growth kinetics analysis *L. rhamnosus* strain was performed in the previously determined optimal conditions of temperature (37°C) and pH (6.0), in MRS broth. Śliżewska et al., 2020 observed comparable lag phase duration when *Lactobacillus* spp., namely, *L. rhamnousus* ŁOCK 1087 and *L. paracasei* ŁOCK 1091 cultivated in the semi-solid fermentation (SSF) medium. Despite the prolonged lag period, the culture of the examined strains on the SSF medium resulted in better cell yields as well as up to two times faster growth rates. These findings were in agreement with Brignone et al. [1], whose research aimed to select a substance for *Lactobacillus* spp. growth enhancement. These show that growth rate is dependent on both strains and the composition of the medium. Additionally, the generation time was related to the strain and culture medium. In both the MRS broth and the SSF medium, *L. pentosus* OCK 1094 showed the quickest cell doubling time, but *L. rhamnosus OCK* 1087 required the longest time (Śliżewska et al., 2020). In conclusion, the findings of the growth kinetics investigation demonstrate a positive effect

for *Lactobacillus* spp. in DF medium with short lag phase compared to glucose medium which has elongated lag phase.

Substrate utilization curve shown in Fig 4.1, where glucose and DF was used as substrate for *L. rhamnosus* ATCC 7469. Result showed that there was sudden decrease in glucose concentration where as in DF medium, bacteria utilised the fiber in steady state which helps in post prandial diabetes. At 0 h, the glucose was 1131.37 mg/L and at 30 h of fermentation the glucose concentration comes to 0.49 mg/L. Similarly, the DF was found to be 0.073% at 0 h and 0.03% at 30 h.




(b)
Dietary fiber concentration (%)
0.08
0.07
0.06
0.05
0.04
0.03
0
5
10
15
20
25
30
Time (h)


(c)
Number of cell [Log CFU/g]
7.5
7.0
6.5
6.0
5.5
Growth kinetics in Glucose medium
0
5
10
15
20
25
30
Time (h)

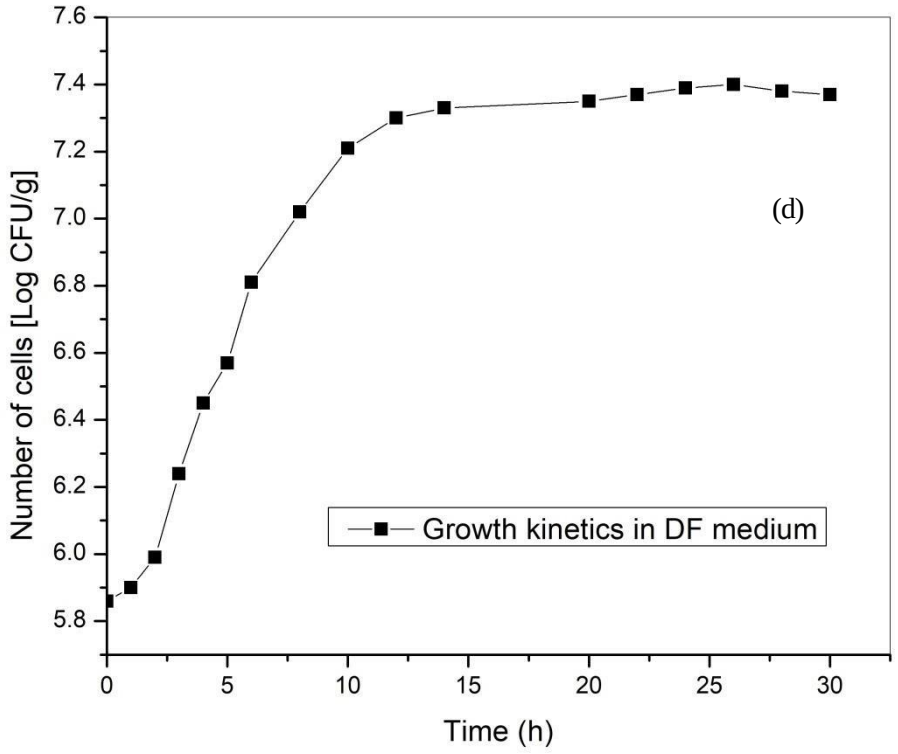


Fig 4.1 The growth kinetics of *Lactobacillus* spp., *L. rhamnosus* ATCC 7469 in glucose (c) and dietary fiber medium (d) for 30 h experiment. The glucose and DF utilization was presented in (a) or (b). Results are presented as the logarithm of colony-forming units per gram [Log CFU/g].

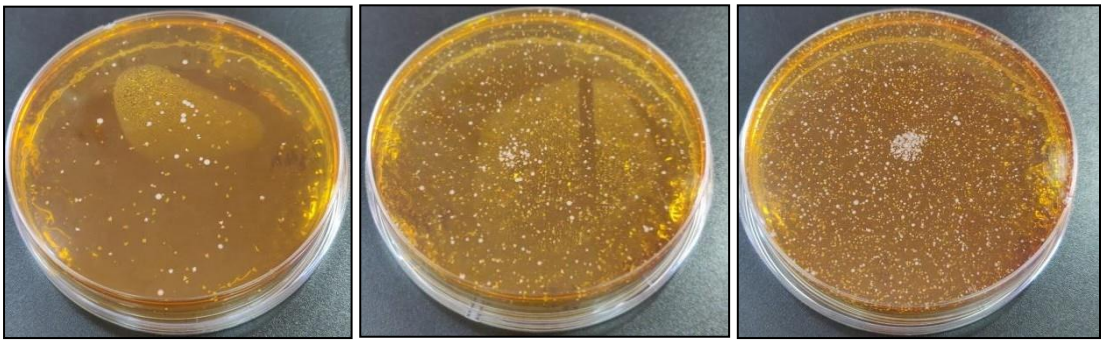


Fig 4.2 Microbial growth of *L. rhamnosus* at 10^{-3}, 10^{-2} and 10^{-1} serial dilution at 1 h during extraction kinetics

4.3.4 Effect on dietary fiber and glucose digestion

In vitro gastrointestinal digestion

For this purpose, the samples were subjected to *in vitro* gastrointestinal conditions where firstly all samples were passes through SSF (Table 4.2) where salivary amylase and lingual lipage digests starch and lipid. As DF does not digest by SSF, further its disintegration continues to G-phase. Salivary amylase denatured once entering to G-phase due to high pH- 2.1. The values of the dietary fiber found gradually decreased from 37 mg to 18 mg with respect to durations in gastro intestinal phase. The glycosidic connections between component monosaccharides and ester linkages can be hydrolyzed by acid at the stomach's extremely low pH (SGF at pH- 2.1). Although pancreatic lipase and other esterases may have modest hydrolytic activity toward methyl esters, O-acetyl esters, and N-acetylamide groups in DF molecules, DF is resistant to hydrolysis by human digestive enzymes. [2]. Likewise, inulin content which was taken as control significantly decreased from 16 mg to 7 mg with respect to time in both G-phase and I-phase. The percentage of DF degradation was higher comparing to I that could be due to hydration property of DF. Depending on its unique hydration characteristic, DF will absorb water and expand to varied degrees as it travels through the stomach and intestine. Following swelling, particulate matter and soluble DF from cell walls (fragments) partially dissolve and enter the digestive juices. In the presence of micro-organism in the samples; DFB and IB, DF and I content significantly decreased from 36 mg to 12 mg and 15 mg to 5 mg respectively (Table 4.8). Probiotic micro-organism *LR* may boosted the hydrolysis of DF and The permeability of the cell wall to digestive fluids, which is SGF and SIF (Table S3, S4), changes when DF is solubilized from the cell wall (fragments), which may result in a decrease in particle size [7, 8] and a change in the surface and structure of the particle. Micro-organism may also help to increase the inter-particles voids and the intra-particles pores and thus causing the hydration and binding properties of the remaining DF and I. Li et al. [12] and Capuano et al. [2] also supported the result where *L. rhamnosus*, the survival rates with simulated gastric juice, simulated intestinal juice, and bile salt were 88.2, 28.03, and 19.4%, respectively could tolerate simulated intestinal and gastric juice (survival counts $>10^6$ CFU/mL) that could be the reason for more degradation of DF.

As observed in **Table 4.7**, glucose concentration in sample DF, had significant increase in both G and I-phase from 25.19±0.6 to 87.05±1.2 and from 16.65±0.5 to 38.6±0.4 µg respectively that could be due to DF used as substrate during its enzymatic hydrolysis. Depending on the sugars used, the glycosidic bond's resistance to acid hydrolysis varies. As a result of the hydrolysis, reducing sugars and pieces of the linear chain of the fiber were released. Microorganism combined with fiber and inulin prone to highest degradation of glucose compared to samples without microorganism as could be possible that glucose was utilised by the microorganism. The breakdown of DF may be attributed to the hydrolysis of compounds with increased time of reaction, probably due to the presence of probiotic microorganism, pepsin (a proteolytic enzyme), gastric lipase and pancreatic juice present in the *in vitro* gastro-intestinal system in **Table 4.3.** The breakdown of macromolecules into their individual monomers, such as sugars, amino acids, peptides, and fatty acids, allows a variety of membrane-bound brush-border transporters to effectively absorb the constituents [4, 2]. These results indicated that presence of micro-organism favors the presence of dietary fiber in the colon to be easily fermented by the bacteria.

Table 4.7 Glucose concentration (µg) in simulated gastrointestinal model

Sample	Gastric phase Glucose concentration (µg)			Intestinal phase Glucose concentration (µg)		
	0 h	1 h	2 h	0 h	1 h	2 h
DF	25.19±0.6^{a}	27.42±0.6^{a}	87.05±1.2^{a}	16.65±0.5^{a}	22.45±0.5^{a}	35.92±0.3^{c}
I	29.65±0.7^{b}	30.98±0.9^{a}	90.34±1.2^{a}	20.6±0.5^{b}	23.44±0.4^{a}	38.6±0.4^{d}
DFB	31.47±0.7^{c}	43.33±1.2^{b}	97.45±1.3^{b}	23.86±0.6^{b}	36.17±0.3^{c}	18.23±0.2^{a}
IB	38.92±0.7^{d}	73.13±1.5^{c}	106.07±1.6^{c}	29.70±0.7^{c}	30.8±0.4^{b}	23.82±0.4^{b}

DF: Pea peel dietary fiber; I: Inulin; DFB: Pea peel dietary fiber with *L. rhamnosus* bacteria; IB: Inulin with *L. rhamnosus* bacteria

Table 4.8 Dietary fiber and inulin content (mg) in simulated gastrointestinal model

Sample	Gastric phase Dietary fiber/ Inulin content (mg)			Intestinal phase Dietary fiber/ Inulin content (mg)		
	0 h	1 h	2 h	0 h	1 h	2 h
DF	37 ± 0.9^{d}	28 ± 0.6^{d}	23 ± 0.3^{d}	23 ± 0.2^{c}	19 ± 0.5^{d}	18 ± 0.3^{d}
I	16 ± 05^{b}	13 ± 0.2^{a}	10 ± 0.5^{a}	10 ± 0.1^{a}	9 ± 0.1^{b}	7 ± 0.1^{b}
DFB	36 ± 0.8^{c}	16 ± 0.3^{c}	13 ± 0.4^{c}	15 ± 0.5^{b}	14 ± 0.2^{c}	12 ± 0.2^{c}
IB	15 ± 0.4^{a}	14 ± 0.3^{b}	11 ± 0.3^{b}	10 ± 0.4^{a}	6 ± 0.1^{a}	5 ± 0.1^{a}

DF: Pea peel dietary fiber; I: Inulin; DFB: Pea peel dietary fiber with *L. rhamnosus* bacteria; IB: Inulin with *L. rhamnosus* bacteria

4.4 Conclusion

According to the results obtained is possible to conclude that pea peel enzymatically modified insoluble dietary fiber (MDF) might be used as prebiotic as it showed a significant positive prebiotic index and relative growth value. The two different prebiotic ingredients used as a growth medium for *L. rhamnosus* ATCC 7469, where DF presented a positive influence over the growth of microbial biomass. With 30 h of fermentation process, it was possible to observe that the prebiotic ingredients that is pea peel DF foster cellular growth of *L. rhamnosus.* Furthermore, under the *in vitro* gastrointestinal digestion model, a significant change was detected in the dietary fiber and glucose fractions in the presence of probiotic *L. rhamnosus.* However, when dietary fiber was subjected to gastro-intestinal phases was more resistant to the enzymes present in the SIF and SGF. These findings emphasise the potential for dietary fiber from pea peels to enter the large intestine, a target organ, and exert possible prebiotic benefits.

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