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Effect of Acid and Oxidation Degradation of Gum Arabic on the Growth of *Lactobacillus* Strains

(Kesan Degradasi Asid dan Pengoksidaan Gam Arab terhadap Pertumbuhan Strain *Lactobacillus*)

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ABSTRACT

Gum arabic consisted of polysaccharides that has the potential to be converted into oligosaccharides through degradation. Thus, this study aimed to determine the effect of gum arabic degradation on the growth of two *Lactobacillus* probiotic strains. Two types of gum arabic (*Acacia senegal* and *Acacia seyal*) were subjected to trifluoroacetic acid (TFA) and oxidation degradation. TFA degradation produced a significantly higher ($p<0.05$) degree of degradation for *A. senegal* (67.6%) and *A. seyal* (62.87%) compared to oxidation method for *A. senegal* (36.49%) and *A. seyal* (39.37%). Thus, TFA degradation was selected as the degradation method. The TFA treated gum arabic was used as growth medium for two types of probiotic strains namely, *Lactobacillus plantarum* and *Lactobacillus reuteri*. Results showed that OD of TFA treated gums from *A. senegal* (1.390) and *A. seyal* (1.330) was significantly higher ($p<0.05$) compared to untreated samples innoculated with *Lactobacillus plantarum*. Similarly, CDM of TFA treated gums innoculated with *Lactobacillus plantarum* for *A. senegal* (1.150 g/L) and *A. seyal* (1.050 g/L) was also significantly higher ($p<0.05$) compared to untreated samples. Using treated gum, the growth (OD and CDM) of *Lactobacillus reuteri* was significantly higher ($p<0.05$) compared to untreated gum. Prebiotic Index (I_{preb}) of both types of treated gum arabic was significantly improved ($p<0.05$) compared to untreated samples. In conclusion, treating gum arabic from both *Acacia senegal* and *Acacia seyal* with TFA produced samples which were more favourable for the growth of *Lactobacillus plantarum* and *Lactobacillus reuteri* strains with significantly increased ($p<0.05$) $I_{preb.}$

Keywords: *Acacia senegal; Acacia seyal; Lactobacillus*; prebiotic; probiotic

ABSTRAK

Gam arab terdiri daripada polisakarida yang berpotensi untuk ditukar menjadi oligosakarida melalui degradasi. Justeru, kajian ini bertujuan untuk menentukan kesan degradasi gam arab terhadap pertumbuhan dua strain probiotik *Lactobacillus*. Dua jenis gam arab (*Acacia senegal* dan *Acacia seyal*) telah melalui proses degradasi menggunakan asid trifluoroasetik (TFA) dan degradasi pengoksidaan. Degradasi TFA menghasilkan darjah degradasi yang lebih tinggi secara signifikan ($p<0.05$) untuk *A. senegal* (67.6%) dan *A. seyal* (62.87%) berbanding kaedah pengoksidaan bagi *A. senegal* (36.49%) dan *A. seyal* (39.37%). Justeru, degradasi TFA telah dipilih sebagai kaedah degradasi. Gam arab terawat TFA digunakan sebagai media pertumbuhan untuk dua strain probiotik iaitu, *Lactobacillus plantarum* dan *Lactobacillus reuteri*. Keputusan menunjukkan OD gam arab terawat menggunakan TFA daripada *A. senegal* (1.390) dan *A. seyal* (1.330) adalah lebih tinggi secara signifikan ($p<0.05$) berbanding sampel tidak terawat yang diinokulasi dengan *Lactobacillus plantarum*. Begitu juga, CDM untuk gam terawat menggunakan TFA yang diinokulasi dengan *Lactobacillus plantarum* untuk *A. senegal* (1.150 g/L) dan *A. seyal* (1.050 g/L) juga adalah lebih

tinggi secara signifikan ($p<0.05$) berbanding sampel tidak terawat. Apabila menggunakan gam terawat, pertumbuhan (OD dan CDM) *Lactobacillus reuteri* adalah lebih tinggi secara signifikan ($p<0.05$) berbanding sampel tidak terawat. Indeks Prebiotik (I_{preb}) bagi kedua-dua gam arab terawat meningkat secara signifikan ($p<0.05$) berbanding sampel tidak terawat. Kesimpulannya, merawat gam arab daripada kedua-dua *Acacia senegal* dan *Acacia seyal* menggunakan TFA menghasilkan sampel yang lebih sesuai untuk pertumbuhan strain *Lactobacillus plantarum* dan *Lactobacillus reuteri* dengan peningkatan I_{preb} yang signifikan ($p<0.05$).

Kata kunci: *Acacia senegal; Acacia seyal; Lactobacillus*; prebiotik; probiotik

Introduction

Gums are complex polysaccharides that possess varying functional properties based on the different chemical compositions and molecular structures (Amid, Mirhosseini & Kostadinović 2012). A lower molecular weight polysaccharide and oligosaccharide will undergo selective fermentation to stimulate intestinal microbiota that provides numerous systemic benefits for the body of the host. As a result of these functional properties, these carbohydrates are often regarded as prebiotics (Gómez et al. 2016).

Oligosaccharides are low molecular saccharide polymers which contain 2–10 monosaccharide units joined by glycosidic bonds and are the main carbohydrate sources in several types of food such as cereals, milk and fruits (Ganzle & Follador 2012). Several types of oligosaccharides such as fructooligosaccharides (FOS), galactooligosaccharides (GOS) and xylooligosaccharides (XOS) has created immense interests among consumers as ingredients for functional foods (Belorkar & Gupta 2016). Nurul et al. (2021) were able to produce oligosaccharides from oil palm mesocarp fibres which showed prebiotic potential when used as growth media for Lactobacillus rhamnosus GR-1 (Mohd et al. 2022).

Acacia gum has high research value and potential economic value due to its abundant inherent nutrients such as protein, fiber and minerals (Osman, Osman & Hassan 2015). Acacia senegal is one type of Acacia gum and possesses excellent rheological properties including increasing the fiber levels without affecting final viscosity (Kiiru, Mahungu & Omwamba 2018). Hammad et al. (2018) reported that gum arabic has the potential to stimulate growth of probiotics based on fecal microbiota from lean individuals. Apart from lean individuals, Ahallil et al. (2020) also showed gum arabic has the potential to support growth of microbiota from obese individuals. Due to these functional properties, it is mainly used as additives in food industry. However, the application of Acacia gum in other areas and further development of its nutrient properties are rarely reported. The main component of Acacia gum is a high molecular weight polysaccharide whose glycoside bonds can be degraded by physical, chemical or biological methods, thereby releasing sugar with lower degree of polymerization. Degradation of Acacia gum polymer may produce novel prebiotic compounds. Although several studies had looked into the degradation of gum arabic (Renard et al. 2014, Sasaki et al. 2022), studies on the utilization of degraded gum arabic as growth media for probiotics has been limited. Gum arabic has been shown to have prebiotic properties (Chundakkattumalayil et al. 2019, Rawi et al. 2021). However, these studies used untreated arabic gum. Degraded arabic gum may produce lower molecule saccharides that increased the growth of probiotics (Ganzle & Follador 2012).

Owing to the complicated structure of polysaccharide in Acacia gum, enzymes proved to be incapable of degrading Acacia gum to produce oligosaccharides (Yoshimi et al. 2017). Ling et al. (2020) reported on the increased functional properties of edible bird nest (EBN) after undergoing hydrolysis. In our efforts to hydrolyze Acacia gum by using glycoside hydrolases, we found that the enzymatic method was not an efficient option. Alternatively, H_2O_2 exhibited strong degradation ability under combined effects of temperature and pressure. Furthermore, trifluoroacetic acid (TFA) may also be able to hydrolyze gum arabic. Thus, in this present study, depolymerized gum arabic prepared using chemical methods was investigated. Production of depolymerized gum arabic from *Acacia senegal* and *Acacia seyal* was achieved either by TFA or H_2O_2. Subsequently, in vitro probiotic growth using the depolymerized gum arabic was investigated.

Materials and Methods

Sample collection and preparation

Acacia senegal and *Acacia seyal* were obtained from a Sudanese market in Khartoum, Sudan. The samples

were in the form of sticky balls of gum. All samples were cleaned from impurities such as bark and sand. All samples were manually cleaned to remove unwanted particles and dried at 50 °C for 3 days. Dried samples were subjected to three grinding steps to produce fine powder form with a maximum mesh size of 0.37 mm. The samples were labelled, placed in containers and kept at room temperature until used.

PREPARATION OF GUM ARABIC FOR DEGRADATION

The gum arabic was first defatted by leaving it in 95% ethanol for 24 h to remove impurities and lipophilic molecules. After that, the precipitate was recovered by centrifugation (3000 g, 15 min). The defatted sample was then diluted 10 times in distilled water and incubated in a thermostatic water-bath at 80 °C for 120 min. The aqueous extract was filtered using a Whatman No. 4 filter paper. The supernatant was concentrated using a rotary vacuum evaporator at 50 °C. Subsequently, 4 volume of ethanol (95%) was added for precipitation followed by incubation at 4 °C in a refrigerator for 24 h. The precipitate was recovered by centrifugation (3000 g, 15 min), and then dissolved in deionized water. Following dialysis (cut off = 10 kDa), a lyophilization step was performed to obtain sample powder (Bouaziz et al. 2016).

DEGRADATION OF GUM ARABIC BY TFA METHOD

One g of water soluble polysaccharide from each type of gum arabic was treated with 100 mL 1.2 M trifluoroacetic acid (TFA) solution for 2.5 h at 85 °C. After the solution was cooled to room temperature, it was centrifuged to obtain the supernatant, while the precipitate was discarded. The supernatant was precipitated overnight by ethanol with a volume ratio of 1:8 (supernatant:ethanol). The precipitate was recovered by centrifugation and oven dried at 40 °C for 6 h. The hydrolyzed sample (0.5 g) was treated with 2 M TFA solution for 2.5 h at 85 °C. After cooling to room temperature, the solution was concentrated to a small volume by rotary evaporation (Zhang et al. 2018). Later, 200 mL of methanol was added into it for the reaction mixture to be evaporated to dryness, then the same amount of methanol was again added and dried by the same method as before, and the procedure was repeated thrice for TFA to be removed and subsequently lyophilized to obtain the treated gum arabic (Dai et al. 2010).

DEGRADATION OF GUM ARABIC BY OXIDATIVE METHOD

An amount of 2.0 g gum arabic was dissolved in 100 mL 0.2M of H_2O_2 solution filled in a thread glass bottle under magnetic stirring. The thread glass bottle was then sealed and put into a high-pressure steam sterilizer for hydrolysis at 120 °C for 2 h. Following the degradation reaction, the reaction mixture was cooled down to room temperature and then incubated in an iced-water bath for 10 min to quench the reaction. The reaction mixture was then filtered and the filtrate was collected and further lyophilized (Liang et al. 2017).

DEGRADATION RATE OF GUM ARABIC CALCULATION

Degradation rate (DR) of *Acacia* gum was calculated according to the yield of reducing sugar (RS) in the degradation product which were defined as the ratio of reducing sugar and the total sugar (TS). Reducing sugar and the total sugar content were determined using dinitro salicylic acid (DNS) colorimetric method and phenol sulfuric acid method.

Determination of total sugar content was done according to the procedure of Dubois et al. (1956). A total of 0.005 g of each sample was dissolved in 100 mL distilled water. One mL of the sample solution was placed in an acid washed tube with addition of 1 mL of 5% phenol solution. This was followed with a quick and direct addition of 5 mL concentrated H_2SO_4 (reagent grade 98.5% with specific gravity of 1.84) and the solution was let to stand for 10 min. Absorbance at 490 nm of the solution was taken using a UV spectrophotometer. Using 10 mg/mL stock glucose solution, standard calibration solutions were prepared in triplicates with concentrations ranging from 0.01 to 0.90 mg/mL.

Determination of reducing sugar content of the samples was performed using 3,5-dinitrosalicylic acid (DNS) method (Saqib & Whitney 2011). DNS reagent was prepared using 1.0 g of dinitrosalicylic acid (DNS) and 30 g of sodium potassium tartrate - Rochelle salt which were added into a 40 mL of 2.0 M sodium hydroxide. Solution was warmed up and mixed well. This was followed with the addition of 100 mL distilled water. In determining reducing sugar content, the analysis was carried out based on the study by Wood and Bhat (1988) with 1 mL of sample solution mixed with 4 mL of DNS reagent. Mixture was incubated in boiling water bath for 5 min and cooled down to room temperature. Absorbance

of the mixture was read at 540 nm using a UV microplate spectrophotometer. Standard curve was set up using 1.00 mg/mL of glucose stock solution by measuring the absorbance with concentrations of 0.10, 0.20, 0.40, 0.60, 0.80 and 1 mg/mL. The degradation rate was calculated using the following equation:

$$DR\% = \frac{RS}{TS} \times 100 \quad (1)$$

where DR is the Degradation Rate; RS is the Reducing Sugar; and TS is the Total Sugar.

GROWTH OF SELECTED PROBIOTIC ON TREATED GUM ARABIC

Two strains of *Lactobacillus c*ulture collection were used; one of them was originally isolated from commercial probiotic capsules (*Lactobacillus reuteri* RC-14). The second one was a widely used commercial probiotic strain *Lactobacillus plantarum* ATCC 8014. Strains were routinely grown in MRS (Biokar Diagnostics, Beauvois, France) at 37 ºC. Overnight cultures (18 h) were used to prepare the bacterial inoculum for the batch culture experiments, which were obtained after collecting cells by centrifugation and suspending them in the same volume of the medium without a carbon source described below.

Uncontrolled-pH batch cultures were performed in a carbohydrate-free basal medium (CFBM) (Salazar et al. 2009). This CFBM contained 2 g/L peptone water (Merck, Darmstadt, Germany), 2 g/L yeast extract (Difco, BD, Biosciences, San Diego, CA), 0.1 g/L sodium chloride (Merck), 0.04 g/L dipotassium phosphate (Merck), 0.04 g/L monopotassium phosphate (Merck), 0.01 g/L magnesium sulphate (Merck), 0.01 g/L hexahydrate calcium chloride (Merck), 2 g/L monosodium carbonate (Merck), 2.5 g/L L-cysteine-HCl (Sigma), 0.5 g/L bile salts (Oxoid Ltd., Basingstoke, Hampshire, UK), 2 mL Tween 80 (Sigma), 0.05 g/L haemin (Sigma) and 10 µL vitamin K1 (Sigma). CFBM were added with 1% (w/v) of three different prebiotic substrates: treated *Acacia senegal,* treated *Acacia seyal* or FOS (as positive control). Each media was distributed into different conical flasks; one additional conical flask was kept without adding any carbon source and used as a negative control. The conical flasks were inoculated with the different *Lactobacillus* at a 1% (v/v). Fermentation was carried out in anaerobic condition at 37 ºC and in a shaker at 170 rpm for 96 h. Samples were collected at fixed incubation periods (0, 6, 12, 24, 48, 72 and 96 h) for analyses. Experiments were carried out three times for each strain.

During fermentation, the hourly samples were withdrawn aseptically for analysis of cell growth. Cell growth was measured by optical density at 600 nm (OD_{600}) and cell dry mass (CDM, expressed in g/L) at 0, 6, 12, 24, 48, 72 and 96 h. About 1 mL of the fermented solution was transferred into pre weighted eppendorf tube and centrifuged at 13000 g for 20 min and then the cells were washed with water for two times. The tubes were dried in oven for 24 h at 100 °C. Following that, the tube was cooled down and weighted again to calculate cell growth. For samples having OD values above 0.9, dilutions were carried out before the final OD determination. Determination of pH in batch cultures was carried out by direct measurement with a pH meter (Crison Instruments S.A., Barcelona, Spain). The culture *medium* was measured in a clean beaker. The pH meter was calibrated with standard buffer solution of pH 7.0 and pH 4.0.

Prebiotic Index (I_{preb}) which refers to the growth of a probiotic in a certain prebiotic relative to its growth in a reference prebiotic was determined as described by Palframan, Gibson and Rastall (2003). In this study FOS was used as a reference prebiotic. I_{preb} for both *Lactobacillus reuteri* RC-14 and *Lactobacillus plantarum* ATCC 8014 was determined. Calculation of I_{preb} was as follows:

$$I_{preb} = \frac{\text{CFU of probiotic in prebiotic carbohydrate}}{\text{CFU of probiotic in reference carbohydrate}} \quad (2)$$

STATISTICAL ANALYSIS

Data were expressed as the means values ± standard deviation. Mean of minimum three measurements were compared by one-way Analysis of Variance. Significant differences between means were determined by Duncan test ($p<0.05$). The software used was SPSS ver.23.

RESULTS AND DISCUSSION

DEGREE OF DEGRADATION OF GUM ARABIC

As can be seen from *Table 1*, both types of gum arabic samples from *Acacia senegal* and *Acacia seyal* were successfully degraded by TFA and H_2O_2. This finding is in agreement with de Moura, Macagnan and da Silva (2015) who reported that gums are degraded to oligosaccharides by hydrolysis though a variety of degradation. In this present study, two methods were used to hydrolyze polysaccharides from *Acacia senegal* and *Acacia seyal* which were acid hydrolysis (TFA) and

oxidation hydrolysis (H_2O_2). It is obvious that, both water soluble polysaccharides were very difficult to depolymerize with H_2O_2. But when treated with TFA, the degradation increased significantly ($p<0.05$) to a value of 62.87 and 67.6% for water soluble polysaccharide from Acacia seyal and Acacia senegal, respectively. Moreover, the acidic degree of degradation rates were similar to those presented by Yan et al. (2018).

Degradation with H_2O_2 gave a dark coloration. This is possibly because when the temperature increased, non-enzymatic browning reaction such as Maillard reaction occured simultaneously and thereafter produces colored substances (Friedman 2002). The degradation rates of both types of gum arabic degraded by H_2O_2 were similar although significantly ($p<0.05$) lower than water soluble polysaccharides degraded by TFA. This was probably owing to the OH which played a critical role in the degradation process which was consumed to a large extent, resulting in decreased oxidative degradation ability (Liang et al. 2017).

GROWTH OF *Lactobacillus plantarum* ATCC 8014 ON TREATED GUM ARABIC

The interaction between sample and incubation period on the growth densities (OD_{600}) and cell dry mass (CDM) of *Lactobacillus plantarum* ATCC 8014 are as shown in Tables 2 and 3. Significant increase ($p<0.05$) of OD was shown from 6 h up to 96 h compared to 0 h for all samples during the fermentation of *Lactobacillus plantarum* ATCC 8014 fed with either treated gum arabic or FOS (Table 2). However, both treated gum arabic alongwith FOS showed a significantly higher ($p<0.05$) increase in OD compared to untreated gum arabic as early as 6 h of fermentation. Comparing between the two types of gum arabic, treated *A. senegal* showed a significantly higher ($p<0.05$) rate of increase in OD compared to *A. seyal* from 6 h upto 72 h of fermentation. No significant difference was observed for OD between treated *A. senegal* and *A. seyal* after 96 h of fermentation. Although FOS showed a significantly higher ($p<0.05$) OD compared to treated gum arabic of both types upto 72 h of ferementation, at 96 h of fermentation, no significant difference in OD was observed between the samples. This showed that the utilization of gum arabic by L. plantarum based on OD values was increased by the TFA treatment to be on par with FOS.

The significant increase ($p<0.05$) of CDM in the fermentation of *Lactobacillus plantarum* ATCC 8014 fed with untreated gum arabic (Table 3) was only recorded after 48 h compared to 0 h. In addition, compared to control, the OD and CDM of *Lactobacillus plantarum* ATCC 8014 fed with untreated gum arabic at 6, 12, 24 and 48 h showed no significant increase, whereas the treated gum arabic showed significant ($p<0.05$) increase in OD and CDM compared to control at 6, 12, 24, 48, 72 and 96 h. Treated gums significantly ($p<0.05$) had higher OD and CDM compared to untreated gums. FOS showed the highest OD density and CDM at 6, 12, 24, 48 and 72 h compared to treated gum, but at 96 h treated gum showed no significant difference in OD and CDM compared to FOS. *Lactobacillus plantarum* ATCC 8014 demonstrated the best growth ($p<0.05$) on treated gum arabic and FOS, but not on the untreated gum arabic.

Degradation of gum arabic may have produced oligosaccharides as seen during TFA degradation of citrus peel pectin (Zhang et al. 2018). Ganzle and Follador (2012) had reported that oligosaccharide plays an important role in the growth of lactobacilli, which may explain the growth behavior of the *Lactobacillus plantarum* ATCC 8014 strains in the current study. Kumar, Rajulapati and Goyal (2020) also observed increased growth of *Lactobacillus plantarum* DM5 in oligosaccharides. In another study, Kaplan and Hutkins (2000) found that *Lactobacillus* strains increased the OD in the presence of oligosaccharide (FOS). Similar growth responses were also shown by *Lactobacillus plantarum* ATCC 8014 strains in the present study. However, the growth in untreated gum arabic was moderate and was significantly lower ($p<0.05$) compared to FOS and treated gum arabic.

The OD and CDM were significantly ($p<0.05$) higher in treated gum arabic than untreated gum arabic. In general, treated gum arabic was better fermented by *Lactobacillus plantarum* ATCC 8014 compared to untreated gum arabic. This might be due to the differences in molecular weight and chemical structure between polysaccharide and oligosaccharide. Pennacchia, Vaughan and Villani (2006) found that *Lactobacillus* strains grew well in the presence of oligosaccharide, but not in polysaccharide. A study by Kaplan and Hutkins (2000) on the fermentation of individual oligomers by *Lactobacillus plantarum* showed better capability of metabolizing the lower molecular weight substances in comparison to the higher molecular weight substances which were not metabolized by these strains.

In addition, some studies carried out on the metabolism of oligosaccharide by *Lactobacillus* strains suggested that the bacteria might have specific enzymatic activities and substrate transport systems that

allowed usage of the specific prebiotic oligosaccharides (Saminathan et al. 2011; Saulnier et al. 2007). The current finding in which *Lactobacillus plantarum* ATCC 8014 could utilize the treated gum arabic suggested the need to further investigate the substrate transport systems in these strains, which has been reported to be more efficient with oligosaccharide. In general, treated gum arabic produced better prebiotic activity than untreated gum arabic.

TABLE 1. Degradation rate (mean ± SD, n =3) of two types of gum arabic treated using trifluoroacetic acid (TFA) or H_2O_2

	Acacia senegal		*Acacia seyal*	
	TFA	H_2O_2	TFA	H_2O_2
Degradation Rate (%)	67.6±3.44[a]	36.49±1.19[c]	62.87±2.09[b]	39.37±1.59[c]

[a-c]: Means with different letters are significantly different ($p<0.05$)

TABLE 2. Mean value of *OD* xduring the fermentation of trifluoroacetic acid (TFA) treated gum arabic at 0, 6, 12, 24, 48, 72 and 96 h inoculated with *Lactobacillus plantarum* ATCC 8014

Incubation Period (h)	Untreated *A. senegal*	Untreated *A. seyal*	Treated *A. senegal*	Treated *A. seyal*	FOS	Control
0	0.015±0.002[q]	0.024±0.002[q]	0.016±0.002[q]	0.029±0.002[q]	0.012±0.001[q]	0.014±0.002[q]
6	0.109±0.030[op]	0.099±0.002[p]	0.554±0.013[h]	0.327±0.009[lm]	0.531±0.010[hi]	0.101±0.012[p]
12	0.161±0.02[nop]	0.201±0.004[n]	0.720±0.080[g]	0.567±0.012[h]	0.988±0.008[e]	0.172±0.006[no]
24	0.285±0.040[m]	0.203±0.004[n]	0.939±0.080[ef]	0.887±0.006[f]	1.200±0.014[c]	0.288±0.007[m]
48	0.390±0.090[kl]	0.419±0.009[jk]	1.110±0.010[d]	1.001±0.010[e]	1.380±0.008[a]	0.349±0.004[lm]
72	0.590±0.020[h]	0.730±0.008[g]	1.280±0.130[b]	1.205±0.020[c]	1.380±0.004[a]	0.447±0.010[jk]
96	0.750±0.030[g]	0.755±0.008[g]	1.390±0.010[a]	1.330±0.350[ab]	1.370±0.003[a]	0.460±0.002[ij]

[a-q] Means with different letters are significantly different ($p>0.05$)

TABLE 3. Mean value of *CDM* (g/L) during the fermentation of trifluoroacetic acid (TFA) treated gum arabic at 0, 6, 12, 24, 48, 72 and 96 h inoculated with *Lactobacillus plantarum* ATCC 8014

Incubation period (h)	Untreated *A. senegal*	Untreated *A. seyal*	Treated *A. senegal*	Treated *A. seyal*	FOS	Control
0	0.000±0.000[q]	0.000±0.000[q]	0.000±0.001[q]	0.000±0.000[q]	0.000±0.000[q]	0.000±0.000[q]
6	0.075±0.03[pq]	0.050±0.070[pq]	0.250±0.070[klm]	0.150±0.070[mno]	0.300±0.000[jkl]	0.075±0.030[pq]
12	0.100±0.001[opq]	0.100±0.000[opq]	0.450±0.070[ghi]	0.350±0.070[ijk]	0.700±0.001[d]	0.100±0.001[opq]
24	0.125±0.350[nop]	0.100±0.001[opq]	0.700±0.001[d]	0.650±0.030[de]	0.950±0.070[bc]	0.150±0.070[mno]
48	0.225±0.030[lmn]	0.225±0.030[lmn]	0.950±0.040[bc]	0.850±0.070[c]	1.150±0.050[a]	0.200±0.001[lmno]
72	0.400±0.001[hij]	0.500±0.001[fgh]	1.050±0.070[ab]	0.950±0.090[bc]	1.150±0.070[a]	0.150±0.060[mno]
96	0.575±0.030[ef]	0.525±0.030[fg]	1.150±0.060[a]	1.050±0.070[ab]	1.150±0.090[a]	0.200±0.001[lmno]

[a-q] Means with different letters are significantly different ($p>0.05$)

GROWTH OF *Lactobacillus reuteri* RC-14 ON TREATED GUM ARABIC

Tables 4 and 5 show the effects of sample type and incubation period on OD density and CDM during the fermentation of *Lactobacillus reuteri* RC-14, respectively. The performance of the treated gum arabic, untreated gum arabic (*Acacia senegal, Acacia seyal*) and FOS to stimulate the growth of *Lactobacillus reuteri* RC-14 were evaluated based on OD_{600} and cell dry mass during 0 h to 96 h of incubation.

The OD gradually increased ($p<0.05$) (Table 4) from 6 h compared to 0 h in *Lactobacillus reuteri* RC-14 fermented with either treated gum, untreated gum or FOS. Compared to control, after 96 h of fermentation, only treated gum arabic showed significantly higher ($p<0.05$) OD, but not FOS or untreated gum arabic. No significant increase of CDM during the fermentation of *Lactobacillus reuteri* RC-14 with either untreated gum or FOS compared with control after each fermentation period (Table 5). However, the CDM showed a significantly higher ($p<0.05$) values with both treated gum arabic compared with FOS or untreated gum after 12 h of fermentation onwards. In the presence of treated gum samples, the growth (OD and CDM) of tested *Lactobacillus reuteri* RC-14 was significantly improved ($p<0.05$). *Lactobacillus reuteri* RC-14 fed with treated gum arabic (treated *Acacia senegal* or treated *Acacia seyal*) as source of carbon showed a significant ($p<0.05$) growth. Gopal, Sullivan and Smart (2001) also reported that in the metabolism of oligosaccharides, monosaccharide and disaccharide by *Lactobacillus* strains were well utilized as growth substrates by *Lactobacillus* strains. Both treated gum samples (*Acacia senegal* or *Acacia seyal*) showed no significant difference between both of them. There was significantly higher ($p<0.05$) growth of *Lactobacillus reuteri* RC-14 in treated gum arabic. This is may be due to the fact that, prebiotic effects of probiotic greatly depends on the degree of polymerisation (Shoaib et al. 2016). It is also possible that degradation of the gum arabic by TFA produced oligosaccharides which has been reported to enhance the growth of Lactobacillus reuteri. A patent using *Lactobacillus reuteri* reported several types of oligosaccharides as its prebiotic (Nestec S.A, Patent WO2016/066763 A1). Oligosaccharides has also been reported to influence the viability of *Lactobacillus reuteri* by increasing membrane integrity (Schwab, Voel & Ganzle 2007).

Untreated gum arabic and FOS did not promote the growth of *Lactobacillus reuteri* based on OD and CDM. This finding is in agreement with the finding of Saminathan et al. (2011) who studied the effect of FOS on the growth of *Lactobacillus reuteri*. Most prebiotic are utilized differently by *Lactobacillus*, because *Lactobacillus* have specific enzymatic activities and substrate transport systems that allowed them to use the specific prebiotic oligosaccharides (Saminathan et al. 2011; Saulnier et al. 2007). Most importantly, treated gum arabic had significantly improved the growth of *Lactobacillus reuteri* RC-14 as compared to untreated gum arabic.

PREBIOTIC INDEX (I_{preb}) OF *Lactobacillus plantarum* ATCC 8014

Table 6 shows the mean I_{preb} of TFA treated gum arabic inoculated with *Lactobacillus plantarum* ATCC 8014. An I_{preb} value higher than 1 indicates that the treated gum arabic has a higher prebiotic effect on *Lactobacillus plantarum* ATCC 8014 compared to FOS. For each of the four samples studied (untreated *A. senegal*, untreated *A. seyal*, treated *A. senegal* and treated *A. seyal*), I_{preb} showed a significant increase ($p<0.05$) with increasing fermentation period. This result is obviously due to the increased growth of *Lactobacillus plantarum* ATCC 8014 as shown in Table 4. The results also suggested that with increasing incubation period, growth of *Lactobacillus plantarum* ATCC 8014 in the four samples increased at a higher rate compared to growth of *Lactobacillus plantarum* ATCC 8014 in FOS.

Comparing between the four samples, there are no significant differences in I_{preb} between untreated samples and treated *A. seyal.* However, both untreated samples showed a significantly lower ($p<0.05$) I_{preb} compared to treated *A. senegal.* However, at 24 h of fermentation, a significantly higher ($p<0.05$) I_{preb} value was observed for both treated samples compared to untreated samples. Subsequently, 96 h of fermentation resulted in a significantly higher ($p<0.05$) I_{preb} for both treated *A. senegal* and *A. seyal* compared to untreated samples. These results further strengthened the indication from previous results (Tables 2 & 3) that TFA treatment of gum arabic increased its prebiotic potential. As suggested, the TFA treatment may have produced oligosaccharides which were more conducive for the growth of *Lactobacilli* (Ganzle & Follador 2012). No significant differences were reported between treated samples of *A. senegal* and *A. seyal* for all fermentation periods up to 96 h.

PREBIOTIC INDEX (I_{preb}) OF *Lactobacillus reuteri* RC-14

Table 7 shows the I_{preb} of untreated and TFA treated gum arabic from *A. senegal* alongwith *A. seyal* using the probiotic *Lactobacillus reuteri* RC-14. Unlike

Lactobacillus plantarum ATCC 8014 (Table 6), increasing the incubation period of *Lactobacillus reuteri* RC-14 did not result in a significant difference in I_{preb} for untreated samples of both *A. senegal* and *A. seyal* gum arabic. Thus, for untreated samples of both *A. senegal* and *A. seyal*, although there was significant increase in OD (Table 4) and CDM (Table 5), the rate of growth did not significantly differ from those of FOS with increasing fermentation period. However, for TFA treated samples of both *A. senegal* and *A. seyal* gum arabic, a significant increase ($p<0.05$) in I_{preb} was observed when incubation period was prolonged from 6 to 12 h. A further increase in incubation period up to 96 h did not produce any significant differences in I_{preb} for both types of gum arabic.

I_{preb} for treated *A. senegal* and *A. seyal* gum arabic was significantly higher ($p<0.05$) compared to untreated samples when inoculated with *Lactobacillus reuteri* RC-14 at all fermentation period used in the study. At 96 h of fermentation, untreated samples were only able to achieve an I_{preb} values of approximately 1 which shows similar prebiotic potential to FOS. However, from 12 h of fermentation period up to 96 h, treated gum arabic was able to achieve an I_{preb} value of more than 2.5 which proved the benefit of TFA treatment of gum arabic in increasing the prebiotic potential relative to FOS. The results (Table 7) also showed no significant differences of I_{preb} between treated *A. senegal* and *A. seyal* as medium for the growth of *Lactobacillus reuteri* RC-14. The higher value of I_{preb} may be attributed to the higher growth of *Lactobacillus reuteri* RC-14 in treated samples due to the degraded gum arabic which may include oligosaccharides as discussed previously.

TABLE 4. Mean value of *OD* during the fermentation of trifluoroacetic acid (TFA) treated gum arabic at 0, 6, 12, 24, 48, 72 and 96 h inoculated with *Lactobacillus reuteri* RC-14

Incubation period (h)	Untreated *A. senegal*	Untreated *A. seyal*	Treated *A. senegal*	Treated *A. seyal*	FOS	Control
0	0.017 ± 0.002^{n}	0.022 ± 0.002^{n}	0.022 ± 0.002^{n}	0.031 ± 0.002^{n}	0.018 ± 0.0007^{n}	0.014 ± 0.002^{n}
6	0.11 ± 0.012^{m}	0.152 ± 0.03^{kl}	0.211 ± 0.01^{hi}	0.227 ± 0.004^{h}	0.167 ± 0.007^{jk}	0.11 ± 0.002^{m}
12	0.123 ± 0.007^{lm}	0.16 ± 0.02^{k}	0.853 ± 0.01^{c}	0.797 ± 0.02^{d}	0.279 ± 0.003^{g}	0.18 ± 0.005^{ijk}
24	0.202 ± 0.012^{hij}	0.227 ± 0.02^{h}	0.947 ± 0.005^{a}	0.872 ± 0.019^{bc}	0.323 ± 0.002^{f}	0.345 ± 0.012^{ef}
48	0.328 ± 0.021^{ef}	0.276 ± 0.01^{g}	0.95 ± 0.019^{a}	0.894 ± 0.028^{b}	0.339 ± 0.002^{ef}	0.343 ± 0.005^{ef}
72	0.335 ± 0.021^{ef}	0.289 ± 0.016^{g}	0.967 ± 0.008^{a}	0.89 ± 0.018^{b}	0.341 ± 0.005^{ef}	0.348 ± 0.009^{ef}
96	0.345 ± 0.021^{ef}	0.364 ± 0.058^{e}	0.958 ± 0.015^{a}	0.891 ± 0.006^{b}	0.334 ± 0.009^{ef}	0.368 ± 0.005^{e}

[a-n] Means with different letters are significantly different ($p>0.05$)

TABLE 5. Mean value of *CDM (*g/L) during the fermentation of trifluoroacetic acid (TFA) treated gum arabic at 0, 6, 12, 24, 48, 72 and 96 h inoculated with *Lactobacillus reuteri* RC-14

Incubation period (h)	Untreated *A. senegal*	Untreated *A. seyal*	Treated *A. senegal*	Treated *A. seyal*	FOS	Control
0	0.00 ± 0.0^{f}	0.00 ± 0.0^{f}	0.00 ± 0.0^{f}	0.00 ± 0.0^{f}	0.00 ± 0.0^{f}	0.00 ± 0.0^{f}
6	0.075 ± 0.03^{ef}	0.05 ± 0.00^{ef}	0.1 ± 0.0^{def}	0.1 ± 0.0^{def}	0.05 ± 0.07^{ef}	0.00 ± 0.0^{f}
12	$0.1\pm0.0d^{ef}$	0.075 ± 0.03^{ef}	0.65 ± 0.07^{bc}	0.6 ± 0.0^{c}	0.1 ± 0.0^{def}	0.1 ± 0.0^{fed}
24	$0.1\pm0.1d^{ef}$	0.15 ± 0.07^{def}	0.75 ± 0.07^{ab}	0.65 ± 0.07^{bc}	0.15 ± 0.07^{def}	0.2 ± 0.0^{de}
48	0.2 ± 0.07^{de}	0.175 ± 0.03^{de}	0.7 ± 0.0^{abc}	0.7 ± 0.0^{abc}	0.1 ± 0.0^{def}	0.25 ± 0.07^{d}
72	0.2 ± 0.0^{de}	0.2 ± 0.00^{de}	0.75 ± 0.07^{ab}	0.75 ± 0.2^{ab}	0.15 ± 0.07^{def}	0.2 ± 0.0^{de}
96h	0.2 ± 0.07^{de}	0.25 ± 0.07^{d}	0.7 ± 0.0^{abc}	0.8 ± 0.1^{a}	0.2 ± 0.0^{de}	0.25 ± 0.07^{d}

[a-f] Means with different letters are significantly different ($p>0.05$)

TABLE 6. Mean values of Prebiotic Index (PI) of trifluoroacetic acid (TFA) treated gum arabic at 0, 6, 12, 24, 48, 72 and 96 h inoculated with *Lactobacillus plantarum* ATCC 8014

Incubation period (h)	Untreated *A. senegal*	Untreated *A. seyal*	Treated *A. senegal*	Treated *A. seyal*
6	0.65^{i}	0.59^{i}	3.32^{abcd}	1.96^{defghi}
12	0.58^{i}	0.72^{hi}	2.58^{bcdef}	2.03^{defghi}
24	0.88^{ghi}	0.63^{i}	2.91^{abcde}	2.75^{abcde}
48	1.15^{fghi}	1.24^{fghi}	3.27^{abcd}	2.95^{abcde}
72	1.73^{efghi}	2.14^{cdefgh}	3.75^{ab}	3.53^{abc}
96	2.25^{cdefg}	2.26^{cdefg}	4.16^{a}	3.98^{ab}

$^{a-i}$: Means with different letters are significantly different ($p<0.05$)

TABLE 7. Mean values of Prebiotic Index (PI) of trifluoroacetic acid (TFA) treated gum arabic at 0, 6, 12, 24, 48, 72 and 96 h inoculated with *Lactobacillus reuteri* RC-14

Incubation period (h)	Untreated *A. senegal*	Untreated *A. seyal*	Treated *A. senegal*	Treated *A. seyal*
6	0.66^{fgh}	0.91^{defg}	1.26^{cd}	1.36^{c}
12	0.44^{h}	0.57^{gh}	3.06^{a}	2.86^{ab}
24	0.63^{fgh}	0.70^{efgh}	2.93^{ab}	2.70^{ab}
48	0.97^{cdefg}	0.81^{efgh}	2.80^{ab}	2.64^{b}
72	0.98^{cdefg}	0.85^{efgh}	2.84^{ab}	2.61^{b}
96	1.03^{cdef}	1.09^{cde}	2.87^{ab}	2.67^{ab}

$^{a-h}$: Means with different letters are significantly different ($p<0.05$)

CONCLUSIONS

In this study, TFA degradation method proved to be more efficient than H_2O_2 oxidative degradation method in degrading gum arabic. Treated gum arabic samples were found to have prebiotic activity with the tested probiotics. Hydrolysis of gum arabic produced higher prebiotic in both types of gum arabic. The present study demonstrated that, treated polysaccharides from both (*Acacia senegal* and *Acacia seyal*) were favorably suited for the growth of the two probiotic *Lactobacillus* strains and were therefore considered as suitable prebiotics for the strains.

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