fermentation

MDPI

Article

Hydrolysis, Microstructural Profiling and Utilization of *Cyamopsis tetragonoloba* in Yoghurt

Majid Hussain [1], Saeed Akhtar [1], Nazia Khalid [2], Muhammad Azam [3,*], Muhammad Waheed Iqbal [4], Tariq Ismail [1], Imran Mahmood Khan [5], Noman Walayat [6], Taha Mehany [7,8], Tuba Esatbeyoglu [9,*] and Sameh A. Korma [10,11]

1 Institute of Food Science & Nutrition, Bahauddin Zakariya University, Multan 60800, Pakistan
2 Department of Food Science & Technology, GC University for Women, Faisalabad 38000, Pakistan
3 National Institute of Food Science and Technology, University of Agriculture, Faisalabad 38000, Pakistan
4 School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, China
5 State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China
6 College of Food Science and Technology, Zhejiang University of Technology, Hangzhou 310014, China
7 Department of Food Technology, Arid Lands Cultivation Research Institute, City of Scientific Research and Technological Applications, Alexandria 21934, Egypt
8 Department of Chemistry, University of La Rioja, 26006 Logroño, Spain
9 Department of Food Development and Food Quality, Institute of Food Science and Human Nutrition, Gottfried Wilhelm Leibniz University Hannover, Am Kleinen Felde 30, 30167 Hannover, Germany
10 Department of Food Science, Faculty of Agriculture, Zagazig University, Zagazig 44519, Egypt
11 School of Food Science and Engineering, South China University of Technology, Guangzhou 510641, China
* Correspondence: muhammadazamfst@yahoo.com (M.A.); esatbeyoglu@lw.uni-hannover.de (T.E.); Tel.: +86-1581-8114-375 (M.A.); +49-5117-6255-89 (T.E.)

Abstract: The present study investigates the hydrolysis, microstructural profiling and utilization of guar gum (*Cyamopsis tetragonoloba*) as a prebiotic in a yoghurt. Guar galactomannans (GG) was purified and partially depolymerized using an acid, alkali and enzyme to improve its characteristics and increase its utilization. The prebiotic potential of hydrolyzed guar gum was determined using Basel and supplemented media. Crude guar galactomannans (CGG), purified guar galactomannans (PGG), base hydrolyzed guar galactomannans (BHGG), acid hydrolyzed guar galactomannans (AHGG) and enzymatic hydrolyzed guar galactomannans (EHGG) were analyzed using scanning electron microscope (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). Yoghurt was prepared with a starter culture and incorporating guar gum, its hydrolyzed forms (0.1, 0.5 and 1%) and *Bifidobacterium bifidum*. The results showed that PHGG significantly improved the viability of *B. bifidum*. SEM revealed a significant change in the surface morphology of guar gum after acidic and enzymatic hydrolysis. Enzymatic hydrolysis developed a well-defined framework within guar gum molecules. The XRD pattern of CGG, PGG and AHGG presented an amorphous structure and showed low overall crystallinity while EHGG and BHGG resulted in slightly increased crystallinity regions. FTIR spectral analysis suggested that, after hydrolysis, there was no major transformation of functional groups. The addition of the probiotic and prebiotic significantly improved the physiochemical properties of the developed yoghurt. The firmness, cohesiveness, adhesiveness and syneresis were increased while consistency and viscosity were decreased during storage. In sum, a partial hydrolysis of guar gum could be achieved using inexpensive methods with commercial significance.

Keywords: fermentation; functional food; prebiotic; guar gum; hydrolysis; *Bifidobacterium bifidum*; yoghurt

Citation: Hussain, M.; Akhtar, S.; Khalid, N.; Azam, M.; Iqbal, M.W.; Ismail, T.; Khan, I.M.; Walayat, N.; Mehany, T.; Esatbeyoglu, T.; et al. Hydrolysis, Microstructural Profiling and Utilization of *Cyamopsis tetragonoloba* in Yoghurt. *Fermentation* **2023**, *9*, 45. https://doi.org/10.3390/fermentation9010045

Academic Editors: Irena Barukcic and Guijie Chen

Received: 29 November 2022
Revised: 24 December 2022
Accepted: 30 December 2022
Published: 4 January 2023

1. Introduction

Native guar gum has beneficial physiological impacts on human health [1–3]. Its incorporation in enteral solutions and food products are limited because of its high viscosity. The viscous nature interferes with the digestion and absorption of nutrients. Due

to the high molecular weight, guar gum is not easily available to beneficial bacteria for their activity. Therefore, moderate hydrolysis is needed to reduce the molecular weight resulting in altered flow attributes in solution and an enhanced prebiotic effect, without disturbing the chemical nature of the gum. Partially hydrolyzed guar gum (PHGG) can be achieved through acid and enzyme hydrolysis, irradiation, microwave and ultra-sonication techniques [4,5]. PHGG is a natural dietary fiber with excellent water solubility. Additionally, PHGG is stable at various pH, pressure and temperature levels exhibiting the same physiological functions as native guar gum [6,7]. PHGG helps to decrease pH in the gut which may enhance the absorption of nutrients. Reider and Moosmang [8] investigated the prebiotic activity of PHGG and recommended its utility to alter the compositional and functional properties of gut microbiota. The crude guar gum (CGG) and PHGG can be characterized through microstructural analysis including scanning electron microscope (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) at the microscopic level [4]. Moreover, SEM can be applied to give information about the surface morphology; hence, it is a well-known powerful technique extensively used to analyze the 'network' characteristic structure of polymers [9]. In XRD phenomena, constructive and destructive interference become visible when molecular and crystalline structures are exposed to X-rays and solid matter can be described as amorphous and crystalline [10]. Both quantitative and qualitative information can be attained using spectroscopy.

Functional foods, including probiotic products, are receiving more attention nowadays due to the awareness of people to the nutrition of food for the promotion of good health and well-being. Due to various beneficial effects, dairy products containing synbiotics (probiotics and prebiotics containing food) are attaining high popularity in this category of foods. *Lacticaseibacillus, Lactobacillus*, *Limosilactobacillus*, *Lapidilactobacillus, Latilactobacillus, Lentilactobacillus, Lactiplantibacillus* and *Bifidobacterium* genera have been reported by different researchers as having potential health benefits [11,12]. *Bifidobacterium bifidum* is one of the most recommended species to be used in food products due to its biological and health benefits. Previous investigations reported that *B. bifidum* can be used as an effective tool to cure irritable bowel syndrome [11,13]. *B. bifidum* has effectively reduced the symptoms of the disease such as increased intestinal absorptivity, imbalanced gut microbiota and hypersensitivity to stress. Lim and Shin [14] reported the immunoregulatory and antimicrobial activity of *Bifidobacterium* genera. *B. bifidum* has the potential to remove biofilm formed by *E. coli*. However, these health benefits are linked with their recommended viability (>10^6 log CFU/mL). The viability can be maintained with encapsulation and increased with the help of prebiotics [15].

PHGG can be used to enhance the growth of *Bifidobacterium* in the gut of human beings. Yoghurt is a fermented milk product praised for its therapeutic and beneficial role. The addition of probiotics and prebiotics can enhance the functional properties of the end product. The addition of PHGG as a prebiotic may have possible beneficial effects on the sensory, textural and rheological properties of yoghurt. It may increase the probiotic count of yoghurt. Keeping in view the significance of PHGG, therefore, the current study was planned to hydrolyze the crude guar gum and applies in yoghurt. To characterize it microstructurally using SEM, XRD and FTIR to explore the differences among various hydrolyzed derivatives and utilize its prebiotic potential to enhance the viable *B. bifidum* count in a developed functional yoghurt model.

2. Materials and Methods

2.1. Procurement of Materials

Crude guar gum (galactomannans) was purchased from Azeem Chemicals (Pvt. Ltd., Faisalabad, Pakistan). Mannanase (EC 3.2.1.78, activity: 0.0002 units/mL) enzyme was obtained from Novozymes (Bagsvaerd, Denmark). All other chemicals and reagents used were acquired from Sigma Aldrich (St. Louis, MO, USA).

2.2. Purification of Guar Gum

Fine powder of crude guar gum (100 g) was dissolved in 2 L of distilled water and allowed to stand for 24 h with intermittent stirring. The gum mucilage was strained with calico to remove any insoluble debris or impurities and precipitated with 500 mL of 96% ethanol. The precipitated gum was re-filtered, washed with diethyl ether and freeze-dried (CHRIST, Alpha 1-4 LSCplus, Osterode am Harz, Germany) at −55 °C. The dried purified gum was milled to a fine powder and checked through a 1 mm sieve [16].

2.3. Hydrolysis of Guar Gum

2.3.1. Acidic Hydrolysis

Guar gum (10 g) was dissolved in 80% aqueous methanol (200 mL) containing HCl (5% w/v). The reaction mixture was heated for 2.5 h at 65 °C. The depolymerized guar gum was neutralized with 1 N NaOH solution and filtered under suction, then, washed with ethanol, freeze-dried and milled to a fine powder and checked using a 1 mm sieve [17].

2.3.2. Alkaline Hydrolysis

Guar gum (5 g) was basically hydrolyzed with a saturated barium hydroxide solution (200 mL) at 100 °C for 8 h. The hydrolyzed gum was neutralized with 1 M H_2SO_4, filtered, freeze-dried and milled to a fine powder and checked by means of a 1 mm sieve [18].

2.3.3. Enzymatic Hydrolysis

Guar gum powder was hydrolyzed with the enzyme mannanase following the procedure of Cheng and Prud'homme [19] with some modifications. Guar powder (1.5 g) was sprinkled slowly onto 198.5 mL of deionized water. The mixture was stirred through a magnetic stirrer during the reaction. A total of 0.04 mg (0.04 units/200 mL) of mannanase enzyme was diluted in 2 mL of 0.1 M sodium acetate/acetic acid buffer solution with pH adjusted to 6 and mixed thoroughly for 60 min. The solution pH was adjusted to 7 using HCl (37%, sp. gravity, 1.19 g/mL). Finally, the polymer solution was transferred to a container and placed for approximately 20–24 h at 25 °C to complete hydration. The mixture was magnetically stirred during the reaction. The guar and enzyme mixture was immediately heated to 100 °C for 20 min to denature the enzyme and stop the reaction. The mixture was filtered, and residues were freeze-dried and ground to a fine powder and checked through a 1 mm sieve.

2.4. Characterization of Guar Gum

2.4.1. Scanning Electron Microscopy (SEM)

Guar gum and its hydrolytic forms were examined using SEM to provide information about the size and shape of particles. Photographic images were recorded with a 30 kV scanning electron microscope (JSM5910, JEOL, Tokyo, Japan) with SEI and EDX detectors (INCA200, Oxford Instruments, Oxford, UK) at low (X1000) and high magnification (X2000) at 10 µm for each guar fractions [20].

2.4.2. X-ray Diffraction

X-ray configurations of guar gum samples were examined using an X-ray Diffractometer (JDX 3532, JEOL, Tokyo, Japan) with CuKα as an anode source. Measurements were carried out with a diffraction angle range of 5–60° and resolution of 0.02° at room temperature (45 kW, 40 mA) [21].

2.4.3. FTIR Analysis

Infrared spectral analysis was performed on a spectrometer (Tensor 27, Bruker, Billerica, MA, USA) under dry air at room temperature. The guar gum was mixed with potassium bromide (1 mg of sample/100 mg of KBr) to improve the transmittance. In this analysis, 75 spectral scans were taken (15 scans/per sample) between 4000 and 400 cm^{-1}

wave number. The scan speed was set at 1 cm/s with 4 cm^{-1} resolution. The spectra were pretreated using baseline correction [22].

2.5. Prebiotic Potential of PHGG

The prebiotic potential of PHGG was assessed following the protocol of Azam [12] with slight modifications. The microbial suspension (*B. bifidum*) was prepared in 0.1 M phosphate buffer solution (pH 7.2). Two types of media were prepared for fermentation. Basal media and PHGG-supplemented media were prepared with *B. bifidum* culture. These formulations were used for the experiment Basal media (BM, 1%: 0%), Basal media and acid hydrolyzed guar gum (BMAH, 1%: 1%), Basal media and basic hydrolyzed guar gum (BMBH, 1%: 1%) and Basal media and enzyme hydrolyzed guar gum (BMEH, 1%: 1%). The fermentation was carried out in anerobic conditions and samples were analyzed after predetermined various time intervals (i.e., 0, 6, 12 and 24 h).

2.6. Yoghurt Manufacturing

Standardized cow milk (Fat 3%) was used for yoghurt manufacturing. Different treatments were made using different concentrations of acid, basic and enzymatically hydrolyzed guar gum (0, 0.5 and 1%) (the viscosity of CGG (18.59 Pa s), AHGG (0.149 Pa s) and EHGG (0.022 Pa s). Purified guar gum (PGG) had a lower viscosity (0.217 Pa s) than SCGG and CGG (1.346 Pa s), and a higher viscosity than the BHGG (0.056 Pa s) *B. bifidum* (1%) (Table 1). The milk was homogenized, pasteurized and cooled to 40 °C. The pasteurized milk was inoculated with a starter culture (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus*). The *B. bifidum* and guar gum were added as per the treatment plan. The samples were incubated at 43 ± 2 °C (pH 4.5) [23].

Table 1. Preparation plan of the developed yoghurt produced with guar gum.

Groups	Control	Guar Gum		Hydrolyzed Guar Gum			*B. bifidum* (%)
		CGG (%)	PGG (%)	AHGG (%)	BHGG (%)	EHGG (%)	
T_o	No GG	-	-	-	-	-	-
T_o'	No GG	-	-	-	-	-	0.001
T_1	-	0.1	-	-	-	-	0.001
T_2	-	0.5	-	-	-	-	0.001
T_3	-	1	-	-	-	-	0.001
T_4	-	-	0.1	-	-	-	0.001
T_5	-	-	0.5	-	-	-	0.001
T_6	-	-	1	-	-	-	0.001
T_7	-	-	-	0.1	-	-	0.001
T_8	-	-	-	0.5	-	-	0.001
T_9	-	-	-	1	-	-	0.001
T_{10}	-	-	-	-	0.1	-	0.001
T_{11}	-	-	-	-	0.5	-	0.001
T_{12}	-	-	-	-	1	-	0.001
T_{13}	-	-	-	-	-	0.1	0.001
T_{14}	-	-	-	-	-	0.5	0.001
T_{15}	-	-	-	-	-	1	0.001

GG; Guar gum, CGG; Crude guar gum, PGG; Purified guar gum, AHG; Acid hydrolyzed guar gum, BHGG; Basic hydrolyzed guar gum, EHGG; Enzymatically hydrolyzed guar gum.

2.7. Physicochemical Analysis of Yoghurt

2.7.1. Viscosity

The viscosity of yoghurt was estimated using a Brookfield LVDVE-230 (Middleboro, MA, USA) viscometer. Apparent viscosity was determined on yoghurt at 10 to 15 °C; yoghurt was stirred for 40 s before viscosity measurement. Spindle number 4 was used for this measurement with a rotation of 10 rpm. Viscometer reading was noted in centipoises (CPS) units and percent torque [23].

2.7.2. Syneresis

The whey released by the yoghurt samples was analyzed by the centrifugation of 5 mL yoghurt at 5000× *g* for 20 min at 4 °C and separated whey was measured after 1 min. The amount of whey separation was expressed as the volume of separated whey per 100 mL of yoghurt [24,25].

2.7.3. Water-Holding Capacity (WHC)

WHC was determined by taking 20 g of yoghurt and centrifuging for 10 min at 669× *g* and 20 °C in the centrifuge of Sigma 3K-30 laboratory centrifuge (Sigma, Louis, MO, USA). The whey expelled was removed and weighed [26].

2.7.4. Texture Analysis

The effect of probiotics and prebiotics on the texture of the synbiotic yoghurt was evaluated by performing the texture profile analysis of yoghurt samples on TA-XT Plus Texture Analyzer (Stable Micro Systems, Godalming, Surrey, UK) using a back extrusion plate Probe P-75 (75 mm) with a few modifications [27]. Texture Exponent 32 software was used to run the texture analyzer. The compression was completed within the container. The tests were run at the settings: pre-test speed (1 mm/s); test speed (0.5 mm/s); post-test speed (1 mm/s); hold time (2 s); the rate for data acquisition (200 pps). The complete profiles of curves were also recorded and the following characteristics were computed: firmness, consistency, cohesiveness and adhesiveness.

2.7.5. pH

An electronic digital type of pH meter (Wandong Medical Co., Ltd. Yangzhou, China) was used for pH determination [28]. A sufficient quantity of the representative sample of yoghurt was taken in a beaker in which electrodes of the pH meter were immersed and readings were recorded after calibrating the instrument.

2.7.6. Titratable Acidity

Acidity was determined by direct titration method no. 947.05 [28]. A well-mixed homogeneous yoghurt sample (9 mL) was taken in a small beaker. Then 1–2 drops of phenolphthalein (1% in 95% *v*/*v* ethanol) solution were added as an indicator. After that, it was titrated against N/10 NaOH until a slight pink color appeared as an endpoint which persisted for 30 s. The percentage of acidity (as lactic acid) was calculated.

2.8. Statistical Analysis

The significance of the results for the dietary treatments was analyzed statistically by computing mean squares and F-values (ANOVA) at 5% probability. Two-factor factorial analysis with a completely randomized design (CRD) was performed for storage data using the software Statistix 8.1 (Tallahassee, FL, USA).

3. Results and Discussion

3.1. SEM of Hydrolyses and Non-Hydrolysed Guar Gum

The SEM micrographs showed that crude guar gum (CGG) had a small rough surface morphology, which is helpful in obtaining the highly viscous aqueous solution. CGG existed in granular form without a cross-linking network between the granules, as shown

in Figure 1. A significant change in appearance was observed in the surface morphology of the guar gum after the hydrolysis process. A clear difference was observed between the crude, purified and hydrolyzed guar gum. A soft structure developed when water molecules were released during the lyophilization of the guar gum solution (Figure 1).

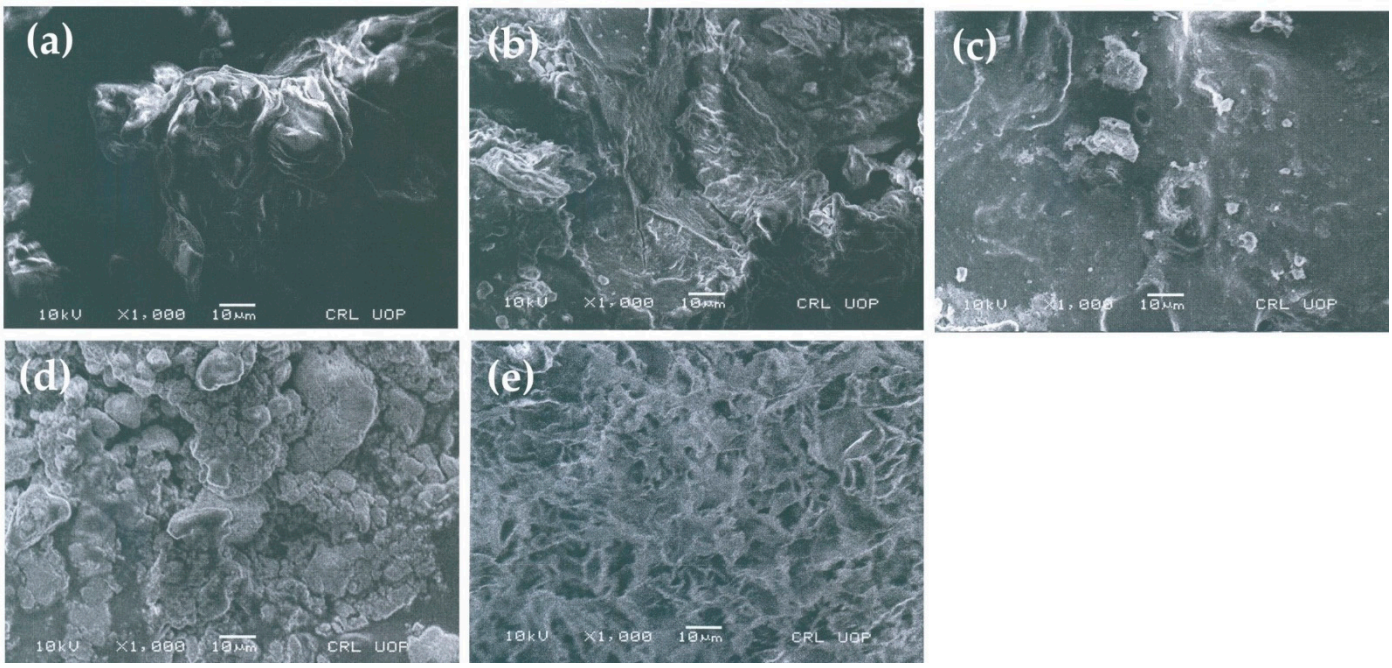

Figure 1. Scanning electron microscope of guar gum: (**a**) Crude guar gum (CGG); (**b**) Purified guar gum (PGG); (**c**) Acid hydrolyzed guar gum (AHGG); (**d**) Basic hydrolyzed guar gum (BHGG); (**e**) Enzymatic hydrolyzed guar gum (EHGG).

The surface of the hydrolyzed samples indicated that morphological changes brought about by hydrolysis as deposits of the hydrolyzed co-polymers were seen as compared to the morphology of CGG. In PGG, it was observed that the surface was rough and had compactness in the molecular structure with the high viscous solution. BHGG displayed the agglomeration of guar particles, compactness and rough surface morphology in their structure after hydrolysis. The base hydrolysis had little effect on the structure of guar gum. The extent of the effect on the BHGG structure was less as compared to AHGG and EHGG, whereas AHGG showed a powdery and fluffy appearance after hydrolysis. Acid hydrolysis of guar gum showed observable changes in its structure which might have been due to a higher metabolic rate yielding the breakage of the galactose and mannose ratio, which was also confirmed due to a reduction in the viscosity of the AHGG aqueous solution as stated elsewhere. In EHGG, the well-defined porous structure was developed as it showed that an excellent interconnected framework was formed by the mannanase enzyme. However, the EHGG showed characteristics of a crosslinking, amorphous and porous structure. Although the structure of EHGG was porous and cross-linked, this type of structure has been known to provide health benefits by increasing the calcium absorption that would be beneficial to the growth of bone cells when added into the consumer's food [29,30]. The actual granular morphology of CGG was lost after the acidic and enzymatic hydrolysis process and transformed into fine, fluffy and well-interconnected morphology, which is advantageous in relation to the acceptable physical behavior of the product in which it would be added, along with imparting a similar rather improved prebiotic endurance [4]. The idea of structural changes of guar gum hydrolyzed in an alkaline environment in the current study is also supported by other studies conducted on the swelling properties of guar gum [31], although the researchers were of the opinion that guar gum was generally found in the granular structure and there was no cross-linking between the granules [20,32].

Obviously, the granular appearance of CGG was lost after the modification of guar and converted into fibrillar morphology [33]. Indeed, it was experienced that a soft structure was produced when water molecules escaped from the guar gum solution during the lyophilization process [34].

3.2. X-ray Diffraction of Hydrolyses and Non-Hydrolysed Guar Gum

XRD configurations (Figure 2) of CGG, PGG and AHGG illustrated an amorphous structure and exhibited low overall crystallinity peaks, observed at a diffraction angle (°2θ) of 20.2, although the crystalline regions of EHGG were slightly higher when seen at an angle (°2θ) at the diffraction of 20.4, 40.2 and 49.5 which is an indication of a slight change in the XRD curve. Basic hydrolysis augmented considerably the crystallinity of the guar gum BHGG at an angle (°2θ) seen at 20.5, 24.1, 26.0, 28.9, 31.4, 33.0, 34.3 and 42.8. A specific peak of guar gum near 2θ = 19.94° was found in the spectrum, which could be due to the weak crystallization or amorphous structure of guar gum [32]. CGG exhibited an amorphous structure in the range of 15–18 at a diffraction angle (°2θ) suggesting that the overall crystallinity in the diffraction band, although being low, increased after cross-linking in guar gum gel. CGG and PHGG (enzymatic hydrolysis) presented an amorphous structure. The former is in line with current findings, whereas the latter showed a bit higher crystallinity which might have been due to the usage of the enzyme, process, method or conditions adopted. GG and PHGG presented less crystallinity at the angle (°2θ) in regions of 20.2 and 72.5. This means that enzymatic hydrolysis of guar gum caused somewhat increased crystallinity regions of PHGG [21]. In another study, the crystallinity index measured for crude guar gum and PHGG was 3.86% and 13.2% accordingly. The treatment of guar gum through an enzymatic process caused an increase in the crystallinity of PHGG [35].

3.3. FTIR Spectroscopy of Hydrolyses and Non-Hydrolyzed Guar Gum

FTIR specific arrangements of CGG, PGG, BHGG, AHGG and EHGG were verified and are summarized in Table 2. In hydrolyzed guar derivatives, spectral peaks ranging from 827.1060 cm^{-1} to 852.8397 cm^{-1} indicated the presence of alkyl halides (C-Cl stretch) which were not present in CGG and PGG. All guar derivatives except BHGG exhibited sharp regions from 1038.6944 cm^{-1} to 1198.8154 cm^{-1}, 1627.7108 cm^{-1} to 1647.7259 cm^{-1}, 2313.9431 cm^{-1} to 2339.6772 cm^{-1} and 2685.6528 cm^{-1} to 2802.8842 cm^{-1} declaring aliphatic amines (C-N stretch), amines (N-H bond), nitriles (C≡N stretch) and aldehydes (H-C=O: C-H stretch), respectively. The sharp peaks for nitro compounds (N-O symmetric stretch) in the spectra of guar derivatives appeared ranging from 1301.7503 cm^{-1} to 1367.5142 cm^{-1} while for aromatics (C-C stretch in a ring) they only appeared in CGG (1559.0875 cm^{-1}) and BHGG (1521.9166 cm^{-1}). The absorbance of nitro compounds (N-O asymmetric stretch) and carboxylic acids appeared in the range from 1501.9015 cm^{-1} to 1507.6201 cm^{-1} and 2611.3110 cm^{-1} to 2694.3551 cm^{-1}, respectively, in guar derivatives except for EHGG. In the spectral array of CGG, PGG and EHGG, peaks were observed in the wavelength ranging from 1719.2085 cm^{-1} to 1782.1130 cm^{-1} and 2851.4924 cm^{-1} to 2911.5377 cm^{-1} that were assigned to ketones (C=O stretch) and alkanes (C-H stretch), respectively. The characteristic absorbance for alkynes was observed in PGG, AHGG and EHGG in the range 2082.3399 cm^{-1} to 2236.7423 cm^{-1}. Another peak around 2356.8330 cm^{-1} to 2379.7074 cm^{-1} was observed in all the spectra except PGG which was possibly due to ammonium ions (N-H). The region of FTIR spectra between 2800 and 3000 cm^{-1} presented C-H stretching modes. The peak in the spectra around 2600 cm^{-1} was due to the OH stretching vibration of the carboxylic acid of polymer and water involved in hydrogen bonding and the spectra around 1700–1850 cm^{-1} were C=O stretching vibrations of the ketone group [21,36].

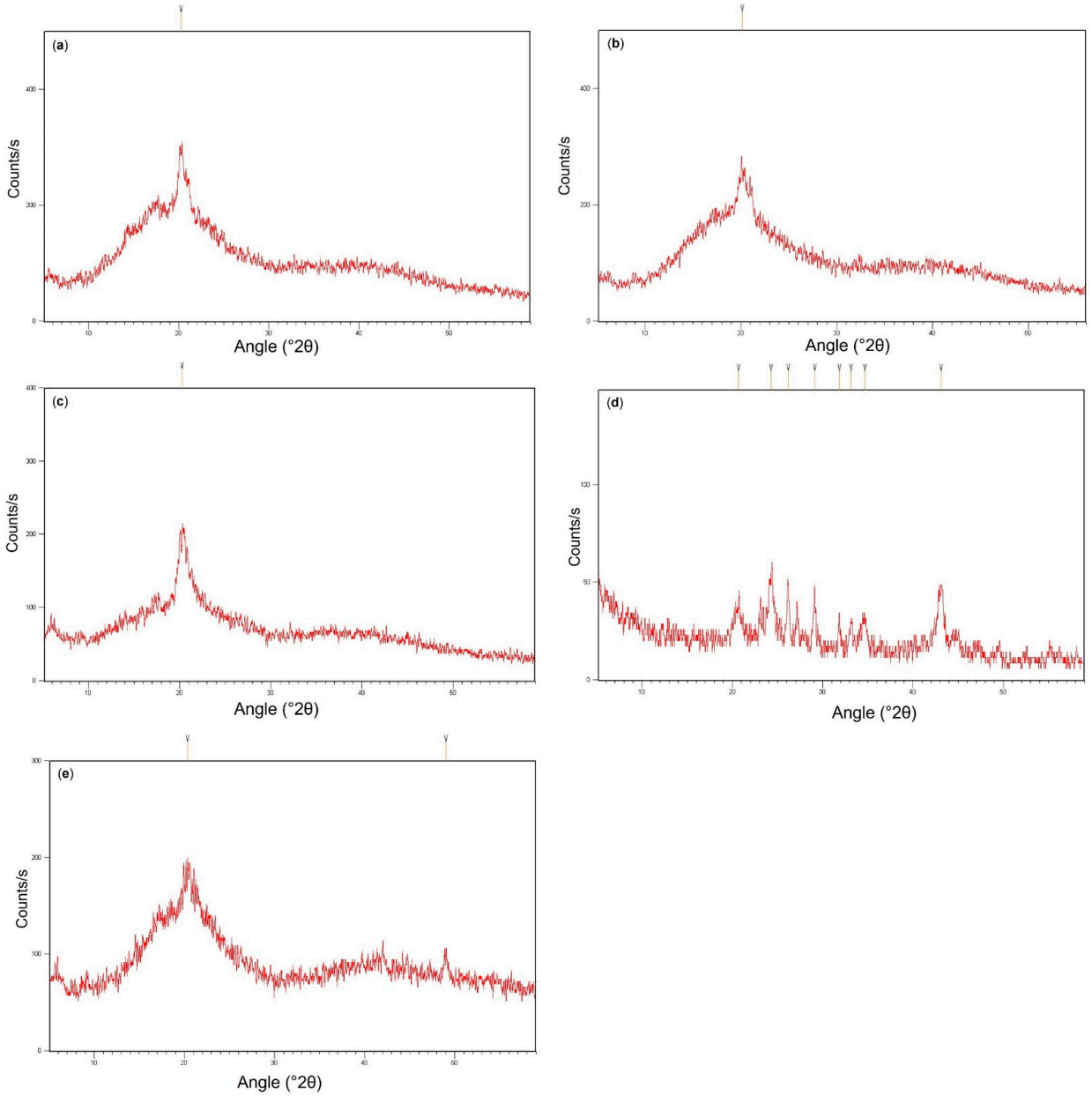

Figure 2. Spectral data of guar gum obtained by X-ray Diffraction (XRD): (**a**) crude guar gum (CGG); (**b**) purified guar gum (PGG); (**c**) acid hydrolyzed guar gum (AHGG); (**d**) basic hydrolyzed guar gum (BHGG); (**e**) enzymatic hydrolyzed guar gum (EHGG).

Table 2. Functional groups evaluation of various guar gums at specific wavenumbers (cm^{-1}) in the infrared spectral region.

Compound	Functional Group	CGG	PGG	AHGG	BHGG	EHGG
C-Cl stretch	Alkyl halides	-	-	852.8397	847.1211	827.1060
C-N stretch	Aliphatic amines	1041.5537	1147.3479	1038.6944	-	1198.8154
N-O symmetric stretch	Nitro compounds	1341.7805	1301.7503	1301.7503	1367.5142	1359.7391
N-O asymmetric stretch	Nitro compounds	1504.7608	1507.6201	1505.7042	1501.9015	-
C-C stretch (in-ring)	Aromatics	1559.0875	-	-	1521.9166	-

Table 2. *Cont.*

Compound	Functional Group	CGG	PGG	AHGG	BHGG	EHGG
N-H bond	Amines	1629.5945	1636.2887	1647.7259	-	1627.7108
C=O stretch	Ketones	1782.1130	1833.5806	-	-	1719.2085
–C≡C– stretch	Alkynes	-	2236.7423	2156.6818	-	2082.3399
C≡N stretch	Nitriles	2313.9435	2325.3807	2313.9431	-	2339.6772
N-H	Ammonium ions	2356.8330	-	2379.7074	2359.6923	2365.4109
O-H stretch	Carboxylic acids	2619.8889	2611.3110	2625.6075	2694.3551	-
H-C=O: C-H stretch	Aldehydes	2685.6528	2780.0098	2802.8842	-	2788.5877
C-H stretch	Alkanes	2911.5377	2851.4924	-	-	2894.3819

CGG, Crude guar gum; PGG, Purified guar gum; AHGG, Acid hydrolyzed guar gum; BHGG, Base hydrolyzed guar gum; EHGG, Enzyme hydrolyzed guar gum.

In PHGG, the sharpening of the absorption band around 1627 cm^{-1} showed its increased association with a water molecule, which could be a reference to its better solubility compared to CGG [32]. The protein content of the samples could have caused the presence of the absorption band at 1650 cm^{-1} which is characteristic of the N-H bending (amide bond) [37]. Additional characteristic absorption bands of guar gum appeared at 1607 cm^{-1} and 1534 cm^{-1} due to C=C stretching vibrations and N-H bending vibrations [36]. Associated water molecules resulted in a band near 1650 cm^{-1} in the spectra. The region around 1400 cm^{-1} due to the CH_2 bending vibration was also detected [21,38–41]. The other key features experienced were the spectral region between 800 and 1200 cm^{-1}, which was due to highly coupled C-C-O, C-OH and C-O-C stretching modes of the polymer backbone [20,42,43]. The region between 500 and 700 cm^{-1} is supposed to be sensitive to changes in crystallinity that are indicative of conformational changes. The crystallinity index for depolymerized guar galactomannan was higher than the native, representing the greater crystallinity of the product, which could be possible due to its smaller size [39,44].

3.4. Prebiotic Potential of PHGG

The effect of guar gum and hydrolyzed guar gum was investigated to improve the viability of *B. bifidum*. The PHGG significantly improved the viability of *B. bifidum* (7.40 ± 0.57 to 9.43 ± 0.21 log CFU/mL) with treatments and time (Table 3). Maximum viability (9.43 ± 0.21 log CFU/mL) was observed for BMEH after 24 h of fermentation and the minimum viability (7.40 ± 0.57 log CFU/mL) was observed for BM at 0 h of fermentation. The supplementation of PHGG in the Basel media improved the growth of *B. bifidum*. This may have been due to the improved availability of guar gum for probiotics. Moderate hydrolysis is needed to reduce the molecular weight resulting in altered flow attributes in solution and an enhanced prebiotic effect, without disturbing the chemical nature of the gum. The change may help to increase the microbial count. The findings of Mudgil [4] are in accordance with our results. They probed the probiotic potential of partially hydrolyzed guar gum and concluded that the partially hydrolyzed guar gum improved its availability for the probiotics.

Table 3. Probiotic potential of partially hydrolyzed guar gum (PHGG).

Time (h)	Basel Media (log CFU/mL)	Basel Media Guar Gum (log CFU/mL)	Basel Media Acid Hydrolyzed Guar Gum (log CFU/mL)	Basel Media Basic Hydrolyzed Guar Gum (log CFU/mL)	Enzyme Hydrolyzed Guar Gum (log CFU/mL)
0	7.40 ± 0.57	7.44 ± 0.06	7.45 ± 0.06	7.48 ± 0.53	7.52 ± 0.37
6	7.61 ± 0.32	7.63 ± 0.22	8.23 ± 0.22	8.35 ± 0.42	8.43 ± 0.62
12	7.90 ± 0.27	7.92 ± 0.19	8.52 ± 0.39	8.71 ± 0.57	9.01 ± 0.79
24	8.10 ± 0.89	8.13 ± 0.29	8.98 ± 0.29	9.04 ± 0.49	9.43 ± 0.21

3.5. pH of Yoghurt Prepared with Hydrolyzed and Non-Hydrolyzed Guar Gum

The statistical results indicated that the pH of yoghurt samples differed highly significantly ($p < 0.01$) for storage days and treatments whereas their interaction (days × treatments) was found to be significant ($p < 0.05$). Data regarding pH depicted that the storage interval exhibited a decreasing trend. The mean value for pH at 0 days of storage was 4.46 and it was reduced to 4.14 after the 28th day of storage on an overall basis (Table 4). The decrease throughout the storage was due to the activity of lactic acid bacteria that convert lactose into lactic acid that adds acidity in the product which inversely decreases the pH. Therefore, a decrease in pH is indicative of an increase in acidity as a function of lactose conversion into lactic acid. The results given in Table 4 indicate that the overall mean for treatment showed a maximum pH value of 4.53 in T_0 followed by 4.31 in $T_{0'}$ and 4.51 in T_2 (0.5% CGG), whereas the lowest value was observed in T_{14} (0.5% EHGG) as 4.10. The control samples showed a higher value of pH, but these are comparable with the experimental treatments showing highly significant differences as presented herein. In the results, values with the same letters indicate non-significant differences whereas different letters are indicating the significant effectiveness of treatments on pH. It is apparent from the results that AHGG (1%) and EHGG (0.5%) showed a lower pH comparatively because of the increased activity of bacteria and due to the increased prebiotic effect of guar gum after hydrolysis. Acid and enzyme hydrolysis of guar gum with reduced chain length and viscosity appeared more acceptable for yoghurt formulation [6,45]. In the case of the interaction, the highest mean value of pH observed was 4.53 in T_0 (control) at 0 days of storage which changed to 4.14 on the 28th day of storage, whereas the lowest pH (4.10) was found in T_{14} (0.5% EHGG) at the 28th day of storage. The pH values obtained in this manuscript are in accordance with the findings of Cruz et al. [46] who reported that the storage time had a significant effect on pH. They documented that a decrease in pH during the storage of yoghurt was a result of the formation of lactic acid by the activity of lactic acid bacteria. The current result is also in accordance with the findings of Mazloomi et al. [47] who conducted a study to examine the attributes of synbiotic yoghurt for up to 14 days.

Table 4. Effect of guar gum and storage time on the pH of probiotic yoghurt.

Treatments	Days of Storage				
	0	**7**	**14**	**21**	**28**
T_0	4.53 ± 0.01^{a}	4.42 ± 0.01^{de}	4.32 ± 0.01^{jk}	4.21 ± 0.02^{tu}	4.14 ± 0.01^{yz}
T_0'	4.51 ± 0.02^{ab}	4.38 ± 0.01^{fg}	4.29 ± 0.02^{mn}	4.22 ± 0.01^{st}	4.16 ± 0.01^{xy}
T_1	4.48 ± 0.01^{ab}	4.37 ± 0.01^{gh}	4.26 ± 0.02^{op}	4.19 ± 0.01^{vw}	4.13 ± 0.01^{za}
T_2	4.46 ± 0.01^{bc}	4.33 ± 0.02^{ij}	4.28 ± 0.02^{no}	4.21 ± 0.01^{tu}	4.18 ± 0.02^{wx}
T_3	4.44 ± 0.02^{cd}	4.36 ± 0.02^{hi}	4.27 ± 0.01^{op}	4.18 ± 0.02^{wx}	4.12 ± 0.01^{ab}
T_4	4.48 ± 0.06^{ab}	4.37 ± 0.01^{gh}	4.32 ± 0.01^{jk}	4.26 ± 0.02^{pq}	4.17 ± 0.01^{wx}
T_5	4.45 ± 0.01^{cd}	4.35 ± 0.01^{hi}	4.31 ± 0.02^{kl}	4.25 ± 0.01^{qr}	4.19 ± 0.01^{vw}
T_6	4.44 ± 0.03^{cd}	4.36 ± 0.02^{gh}	4.33 ± 0.01^{ij}	4.27 ± 0.01^{op}	4.20 ± 0.01^{uv}
T_7	4.44 ± 0.03^{cd}	4.35 ± 0.02^{hi}	4.29 ± 0.01^{mn}	4.20 ± 0.01^{uv}	4.11 ± 0.02^{bc}
T_8	4.43 ± 0.01^{cd}	4.33 ± 0.01^{ij}	4.28 ± 0.02^{no}	4.21 ± 0.01^{tu}	4.12 ± 0.02^{ab}
T_9	4.40 ± 0.02^{ef}	4.32 ± 0.01^{jk}	4.28 ± 0.01^{no}	4.22 ± 0.02^{st}	4.14 ± 0.01^{yz}
T_{10}	4.51 ± 0.02^{ab}	4.34 ± 0.01^{ij}	4.30 ± 0.01^{lmn}	4.24 ± 0.01^{rs}	4.19 ± 0.01^{vw}
T_{11}	4.47 ± 0.01^{bc}	4.35 ± 0.02^{hi}	4.28 ± 0.01^{no}	4.19 ± 0.01^{vw}	4.11 ± 0.01^{bc}
T_{12}	4.46 ± 0.01^{bc}	4.34 ± 0.01^{ij}	4.27 ± 0.02^{op}	4.18 ± 0.0^{wx}	4.12 ± 0.01^{ab}
T_{13}	4.47 ± 0.02^{bc}	4.32 ± 0.01^{jk}	4.29 ± 0.01^{mn}	4.20 ± 0.01^{uv}	4.13 ± 0.02^{za}
T_{14}	4.44 ± 0.02^{cd}	4.33 ± 0.02^{ij}	4.25 ± 0.01^{qr}	4.16 ± 0.01^{xy}	4.10 ± 0.02^{c}
T_{15}	4.43 ± 0.02^{cd}	4.32 ± 0.01^{jk}	4.28 ± 0.01^{no}	4.19 ± 0.02^{vw}	4.11 ± 0.01^{bc}

The values are mean ± SD ($n = 3$); Means with different letters differed significantly at ($p \leq 0.05$). Comparisons are made within the column for each concentration of guar fractions and in a row for storage to evaluate the pH effects. (Overall treatment mean; Max. value = 4.32, Min. value = 4.25); LSD value days = 0.0063, LSD value treatments = 0.0118, LSD value interactions (days × treatments) = 0.0264; Control: (T_0, T_0'; without guar gum), CGG: Crude guar gum; (T_1, 0.1%; T_2, 0.5%; T_3, 1%); PGG: Purified guar gum; (T_4, 0.1%; T_5, 0.5%; T_6, 1%), AHGG: Acid hydrolyzed guar gum; (T_7, 0.1%; T_8, 0.5%; T_9, 1%), BHGG: Base hydrolyzed guar gum; (T_{10}, 0.1%; T_{11}, 0.5%; T_{12}, 1%), EHGG: Enzyme hydrolyzed guar gum; (T_{13}, 0.1%; T_{14}, 0.5%; T_{15}, 1%).

They observed a substantial decrease in pH (6.61 to 4.48) during storage as a function of an increase in acidity. Recently, Prasanna and Grandison [48] also reported a decrease in pH with the passage of time which supports the results obtained in this manuscript. It is therefore presented through the results that the pH in the study reduced with the passage of time and it was altered due to the treatments of guar gum applied for the production of yoghurt as a prebiotic. Among the treatments, EHGG and AHGG showed a good relation to the stability of the product indicating a good combination of guar gum as a prebiotic with *Bifidobacterium* as a probiotic combination and the steady change in pH seemed to be a more stable product.

3.6. Acidity of Yoghurt Prepared with Hydrolyzed and Non-Hydrolyzed Guar Gum

The statistical results exhibited a highly significant ($p < 0.01$) effect on acidity due to storage days and treatments whereas their interaction (days × treatments) was found to be significant ($p < 0.05$). Data illustrated that the storage time presented a highly significant influence on acidity with an increasing trend. The mean value for acidity at 0 days of storage was 0.944% and it increased to 1.17% after the 28th day of storage on an overall basis (Table 5).

Table 5. Effect of guar gum and storage time on the acidity (%) of probiotic yoghurt.

	Days of Storage				
Treatments	**0**	**7**	**14**	**21**	**28**
T_0	1.040 ± 0.010 ^{ij}	1.047 ± 0.006 ^{hi}	1.077 ± 0.035 ^{fg}	1.110 ± 0.050 ^{ef}	1.150 ± 0.070 ^{cd}
T_0'	0.980 ± 0.040 ^{qr}	1.043 ± 0.005 ^{hi}	1.067 ± 0.015 ^{fg}	1.090 ± 0.050 ^{fg}	1.123 ± 0.050 ^{de}
T_1	0.940 ± 0.010 ^{uv}	0.993 ± 0.005 ^{mn}	1.067 ± 0.050 ^{fg}	1.083 ± 0.010 ^{fg}	1.250 ± 0.015 ^{a}
T_2	0.770 ± 0.030 ^{z}	0.773 ± 0.030 ^{vw}	0.933 ± 0.035 ^{hi}	1.043 ± 0.025 ^{hi}	1.047 ± 0.025 ^{hi}
T_3	0.940 ± 0.010 ^{uv}	1.020 ± 0.010 ^{jk}	1.037 ± 0.015 ^{ij}	1.043 ± 0.005 ^{hi}	1.147 ± 0.046 ^{cd}
T_4	0.993 ± 0.015 ^{mn}	1.010 ± 0.010 ^{kl}	1.060 ± 0.040 ^{gh}	1.060 ± 0.040 ^{gh}	1.093 ± 0.006 ^{fg}
T_5	0.950 ± 0.036 ^{tu}	0.980 ± 0.030 ^{qr}	1.020 ± 0.020 ^{jk}	1.047 ± 0.006 ^{hi}	1.090 ± 0.040 ^{fg}
T_6	0.897 ± 0.045 ^{xy}	1.013 ± 0.025 ^{kl}	1.013 ± 0.015 ^{ij}	1.050 ± 0.020 ^{hi}	1.243 ± 0.015 ^{ab}
T_7	0.990 ± 0.010 ^{op}	1.000 ± 0.010 ^{lm}	1.020 ± 0.020 ^{jk}	1.087 ± 0.015 ^{fg}	1.133 ± 0.025 ^{de}
T_8	0.910 ± 0.010 ^{wx}	0.973 ± 0.035 ^{rs}	1.033 ± 0.015 ^{ij}	1.030 ± 0.030 ^{jk}	1.193 ± 0.015 ^{bc}
T_9	0.990 ± 0.020 ^{op}	1.040 ± 0.010 ^{ij}	1.097 ± 0.005 ^{fg}	1.123 ± 0.020 ^{de}	1.207 ± 0.035 ^{ab}
T_{10}	0.897 ± 0.015 ^{y}	0.987 ± 0.005 ^{qr}	1.040 ± 0.010 ^{ij}	1.093 ± 0.035 ^{fg}	1.280 ± 0.036 ^{a}
T_{11}	0.933 ± 0.057 ^{vw}	0.980 ± 0.005 ^{pq}	1.010 ± 0.010 ^{kl}	1.040 ± 0.010 ^{ij}	1.060 ± 0.020 ^{gh}
T_{12}	0.960 ± 0.020 st	0.993 ± 0.015 ^{mn}	0.997 ± 0.005 ^{mn}	1.010 ± 0.020 ^{kl}	1.237 ± 0.045 ^{ab}
T_{13}	0.960 ± 0.010 st	0.977 ± 0.015 ^{qr}	0.990 ± 0.010 ^{op}	0.997 ± 0.015 ^{mn}	1.270 ± 0.010 ^{a}
T_{14}	0.987 ± 0.012 ^{qr}	1.007 ± 0.005 ^{kl}	1.030 ± 0.010 ^{jk}	1.080 ± 0.010 ^{fg}	1.153 ± 0.005 ^{bc}
T_{15}	0.930 ± 0.026 ^{vw}	0.973 ± 0.020 ^{rs}	1.007 ± 0.015 ^{kl}	1.193 ± 0.045 ^{ab}	1.233 ± 0.015 ^{ab}

The values are mean ± SD (n = 3); Means with different letters differed significantly at ($p \leq 0.05$). Comparisons are made within the column for each concentration of guar fractions and in a row for storage to evaluate the pH effects. (Overall treatment mean; Max. value = 4.32, Min. value = 4.25); LSD value days = 0.0063, LSD value treatments = 0.0118, LSD value interactions (days x treatments) = 0.0264; Control: (T_0, T_0'; without guar gum), CGG: Crude guar gum; (T_1, 0.1%; T_2, 0.5%; T_3, 1%); PGG: Purified guar gum; (T_4, 0.1%; T_5, 0.5%; T_6, 1%), AHGG: Acid hydrolyzed guar gum; (T_7, 0.1%; T_8, 0.5%; T_9, 1%), BHGG: Base hydrolyzed guar gum; (T_{10}, 0.1%; T_{11}, 0.5%; T_{12}, 1%), EHGG: Enzyme hydrolyzed guar gum; (T_{13}, 0.1%; T_{14}, 0.5%; T_{15}, 1%).

The increase in acidity during the storage period was an effect of lactic acid bacteria that convert lactose into lactic acid. The overall means for treatment showed the highest acidity value of 1.09% in T_9 (1% AHGG) followed by 1.09% in T_0 and 1.067% in T_1 (0.1% CGG) and T_{15} (1% EHGG), whereas the lowest value was observed in T_2 (0.5% CGG) as 0.913%. The control samples showed a relatively higher value of acidity, but these were comparable with the experimental treatments showing highly significant differences as presented herein. In the results, values with the same letters indicate non-significant differences whereas different letters indicate the significant effects of treatments on acidity. It is apparent from the results that AHGG (1%) and EHGG (0.5%) showed more acidity comparatively because of the increased activity of bacteria and due to the increased prebiotic effect of guar gum after hydrolysis. As far as interaction is concerned, the highest mean value of acidity observed was 1.28% in T_{10} (0.1% BHGG) on the 28th day, whereas

the lowest acidity (0.77%) was found in T_2 (0.5% CGG) on 0 days of storage. The findings of the current study are in accordance with the outcomes of Shaghaghi and Pourahmad [49] who reported an increase in acidity during storage when studying the effect of prebiotic incorporation on the quality of synbiotic yoghurt. Similarly, in another study, Khalifa and Elgasim [50] found that the acidity increased with the increase in the storage interval while evaluating the application of stabilizers in yoghurt production during 10 days of storage. Fadela and Abderrahim [51] also reported a similar finding while conducting studies on the use of lactic acid strains in yoghurt manufacture. Acidity is the reverse of pH, so some researchers correlated the effect of pH with acidity. As the pH of the sample decreased, acidity will increase resulting in a more bitter taste and increased whey separation [52]. Karaca [53] studied the effect of different prebiotic stabilizers and types of molasses on different characteristics of probiotic set yoghurt and reported an increase in acidity with the passage of time.

3.7. Syneresis of Yoghurt Prepared with Hydrolyzed and Non-hydrolyzed Guar Gum

From the current findings, it was noticed that syneresis differed highly significantly ($p < 0.01$) among storage days and treatments whereas their interaction (days × treatments) was found to be significant ($p < 0.05$). Data depicted (Table 6) that the storage time had a highly significant influence on syneresis with an increasing trend with the passage of time.

Table 6. Effect of guar gum and storage time on syneresis (%) of probiotic yoghurt prepared from hydrolyzed and non-hydrolyzed guar gum.

Treatments	Days of Storage				
	0	7	14	21	28
T_0	42 ± 0.02^{mn}	56 ± 0.03^{fg}	70 ± 0.02^{de}	73 ± 0.02^{ab}	80 ± 0.03^{a}
T_0'	46 ± 0.02^{op}	48 ± 0.02^{gh}	60 ± 0.02^{bc}	65 ± 0.21^{ab}	70 ± 0.05^{ab}
T_1	40 ± 0.03^{v}	60 ± 0.02^{lm}	66 ± 0.14^{ij}	70 ± 0.02^{fg}	72 ± 0.02^{ef}
T_2	60 ± 0.01^{lm}	70 ± 0.02^{fg}	75 ± 0.24^{jk}	82 ± 0.02^{bc}	85 ± 0.03^{ab}
T_3	58 ± 0.02^{no}	64 ± 0.02^{fg}	65 ± 0.07^{ef}	78 ± 0.15^{bc}	80 ± 0.01^{ab}
T_4	50 ± 0.05^{qr}	58 ± 0.73^{mn}	60 ± 0.15^{lm}	65 ± 0.15^{jk}	82 ± 0.02^{ab}
T_5	55 ± 0.05^{pq}	68 ± 0.02^{fg}	78 ± 0.09^{cd}	80 ± 0.5^{ab}	83 ± 0.01^{a}
T_6	56 ± 0.04^{uv}	70 ± 0.04^{no}	73 ± 0.03^{ef}	78 ± 0.02^{ef}	80 ± 0.02^{ab}
T_7	44 ± 0.02^{tu}	52 ± 0.02^{no}	60 ± 0.02^{hi}	65 ± 0.08^{fg}	80 ± 0.03^{ab}
T_8	43 ± 0.01^{v}	50 ± 0.01^{pq}	60 ± 0.02^{kl}	70 ± 0.15^{de}	80 ± 0.03^{ab}
T_9	48 ± 0.01^{uv}	58 ± 0.02^{lm}	65 ± 0.01^{gh}	70 ± 0.24^{fg}	74 ± 0.07^{de}
T_{10}	40 ± 0.02^{v}	54 ± 0.25^{op}	66 ± 0.02^{ij}	70 ± 0.03^{fg}	80 ± 0.01^{ab}
T_{11}	52 ± 0.02^{qr}	70 ± 0.12^{lm}	77 ± 0.02^{ij}	80 ± 0.10^{fg}	84 ± 0.02^{ab}
T_{12}	50 ± 0.02^{st}	60 ± 0.03^{rs}	66 ± 0.01^{lm}	70 ± 0.5^{jk}	80 ± 0.12^{fg}
T_{13}	44 ± 0.02^{rs}	56 ± 0.15^{mn}	67 ± 0.01^{jk}	70 ± 0.11^{fg}	80 ± 0.12^{de}
T_{14}	40 ± 0.01^{uv}	52 ± 0.02^{qr}	62 ± 0.02^{lm}	65 ± 0.09^{fg}	80 ± 0.02^{ab}
T_{15}	42 ± 0.02^{v}	60 ± 0.10^{pq}	68 ± 0.01^{lm}	70 ± 0.02^{jk}	74 ± 0.01^{ab}

The values are mean ± SD ($n = 3$); Means with different letters differ significantly at ($p \leq 0.05$). Comparisons are made within the column for each concentration of guar fractions and in a row for storage to evaluate the pH effects. (Overall treatment mean; Max. value = 4.32, Min. value = 4.25); LSD value days = 0.0063, LSD value treatments = 0.0118, LSD value interactions (days x treatments) = 0.0264; Control: (T_0, T_0'; without guar gum), CGG: Crude guar gum; (T_1, 0.1%; T_2, 0.5%; T_3, 1%); PGG: Purified guar gum; (T_4, 0.1%; T_5, 0.5%; T_6, 1%), AHGG: Acid hydrolyzed guar gum; (T_7, 0.1%; T_8, 0.5%; T_9, 1%), BHGG: Base hydrolyzed guar gum; (T_{10}, 0.1%; T_{11}, 0.5%; T_{12}, 1%), EHGG: Enzyme hydrolyzed guar gum; (T_{13}, 0.1%; T_{14}, 0.5%; T_{15}, 1%).

The mean value for syneresis at 0 days of storage was 47.4% and it increased to 79.1% on the 28th day of storage on an overall basis. The increase in syneresis may have been due to the activity of the lactic acid bacteria and *B. bifidum*. Increased whey separation was attributed to an unstable and excessive rearrangement of the weak network of the gel. The results indicated that the overall mean for treatment showed maximum syneresis 74.2% in T_2 (0.5% CGG) followed by 72.8% in T_5 (0.5% PGG), 72.6% in T_{11} (0.5% BHGG) and 69.4% in T_3 (1% CGG), whereas the lowest value was observed in T_{14} (0.5% EHGG) as 59.8%. The results on an overall basis indicated that syneresis increased in controlled as well as treated samples, particularly in relation to T_7, T_8, T_{13}, T_{14} and T_{15}. This indicates the comparative

quality of yoghurt texture and body formation but additionally with positive trends for the objectives taken into consideration, e.g., the acceptable symbiotic relationship of probiotics and prebiotics that will ultimately increase probiotic benefits to the consumer [6,45]. The highest mean value of syneresis for interaction (days × treatments) observed was 85% in T_2 (0.5% CGG) at the 28th day of storage, whereas the lowest syneresis (40%) was found in T_1 (0.1% CGG), T_{10} (0.1% BHGG), and T_{14} (0.5% EHGG) at the start of the storage. In another study, conducted by Brennan and Tudorica [27] various samples of yoghurt containing PHGG exhibited a significant reduction in syneresis as compared to with the control yoghurt having low fat ($p < 0.001$), whereas they calculated that increasing the levels of PHGG in the yoghurt preparations gave rise to a reduction in the syneresis of low-fat yoghurt, bringing it to levels comparable to the full-fat control yoghurt specifically when the levels of addition were used above 2%. The incorporation of thickeners significantly ($p < 0.001$) decreased the syneresis as compared to the control yoghurt. Moreover, yoghurt produced with an increased level of gelatin exhibited the lowest syneresis values. In a different study, the syneresis of yoghurt samples was measured at 4 °C. The results showed that samples with gums had less syneresis during storage. Samples containing xanthan gum at a level of 0.01% demonstrated high resistance to syneresis throughout storage [54].

3.8. Water-Holding Capacity (WHC) of the Yoghurt Prepared with Hydrolyzed and Non-Hydrolyzed Guar Gum

The statistical results indicated that WHC differed highly significantly ($p < 0.01$) among storage days and treatments, whereas their interaction (days × treatments) was found to be significant ($p < 0.05$). The results depicted that with the passage of storage time WHC decreased (Table 7). The mean value for WHC at 0 days of storage was 69.1%; later on, it was reduced to 38.2% on the 28th day of storage on an overall basis. The decrease in WHC may have been due to the activity of the lactic acid bacteria and *B. bifidum* and as an effect of increased acidity during storage. The results given in Table 7 depicted that the overall mean for treatment showed a maximum WHC of 66.1% in T_9 (1% AHGG) followed by 65.6% in T_{14} (0.5% EHGG), 63.9% in T_8 (0.5% AHGG) and 60.1% in T_{13} (0.1% EHGG), whereas the lowest value was observed in T_2 (0.5% CGG) as 39.2%.

Table 7. Effect of guar gum and storage time on the water-holding capacity (%) of probiotic yoghurt.

Treatments	Days of Storage				
	0	7	14	21	28
T_o	68.67 ± 0.76^{mn}	68.11 ± 0.11^{no}	64.33 ± 0.61^{st}	51 ± 0.5^{yz}	43.62 ± 0.38^{gh}
T_o'	71.03 ± 1.27^{jk}	66.75 ± 0.25^{pq}	60.6 ± 0.6^{v}	50.9 ± 0.45^{yz}	40.57 ± 0.33^{i}
T_1	73.98 ± 0.49^{d}	72.5 ± 0.5^{hi}	58.05 ± 0.05^{w}	46.33 ± 0.15^{cd}	42.4 ± 0.4^{h}
T_2	32.5 ± 0.5^{m}	38.14 ± 0.144^{k}	68.52 ± 0.66^{mn}	28.52 ± 0.49^{op}	28.4 ± 0.4^{op}
T_3	74.5 ± 0.35^{cde}	68.05 ± 0.05^{no}	46.5 ± 0.5^{bcd}	38.27 ± 0.27^{k}	34.67 ± 0.21^{l}
T_4	73.91 ± 0.41^{c}	72.73 ± 0.11^{ef}	71.98 ± 0.40^{w}	43.28 ± 0.06^{ab}	36.04 ± 0.14^{de}
T_5	42.3 ± 0.15^{h}	44.69 ± 0.1^{fg}	44.65 ± 0.15^{fg}	26.8 ± 0.4^{q}	39.9 ± 0.45^{ij}
T_6	72.8 ± 0.36^{fg}	65.02 ± 0.02^{rs}	63.36 ± 0.12^{tu}	40.3 ± 0.1^{ij}	30.48 ± 0.15^{n}
T_7	80 ± 2^{b}	70.18 ± 0.18^{kl}	50.87 ± 0.175^{z}	46.69 ± 1.04^{bc}	30.14 ± 0.03^{n}
T_8	82.33 ± 0.38^{a}	69.03 ± 0.03^{lm}	62.68 ± 0.18^{u}	57.84 ± 0.34^{w}	47.8 ± 0.4^{ab}
T_9	83.07 ± 0.07^{a}	69.65 ± 0.05^{kl}	66 ± 0.25^{qr}	63.37 ± 0.07^{tu}	48.49 ± 0.49^{a}
T_{10}	73.5 ± 0.5^{b}	68.68 ± 0.18^{cd}	62.21 ± 0.1^{cde}	52.34 ± 0.34^{x}	38.96 ± 0.46^{fg}
T_{11}	39.48 ± 0.1^{no}	40.1 ± 0.1^{ij}	54.94 ± 0.05^{rs}	35.29 ± 0.06^{l}	30.87 ± 0.30^{n}
T_{12}	68.41 ± 0.02^{mno}	64.34 ± 0.04^{st}	58.56 ± 0.06^{w}	45.29 ± 0.21^{def}	27.17 ± 0.17^{pq}
T_{13}	75.5 ± 0.16^{de}	73.3 ± 0.3^{ghi}	58.77 ± 0.09^{ij}	47.34 ± 0.34^{gh}	45.5 ± 0.25^{l}
T_{14}	80.8 ± 0.4^{ef}	75.04 ± 0.06^{mn}	74.21 ± 0.21^{u}	53.4 ± 0.4^{xy}	44.6 ± 0.3^{jk}
T_{15}	74.46 ± 0.21^{cd}	67.5 ± 0.15^{op}	62.84 ± 0.32^{u}	45.21 ± 0.02^{ef}	38.96 ± 0.31^{jk}

Means with different letters differ significantly at ($p \leq 0.05$). Comparisons are made within the column for each concentration of guar fractions and in a row for storage to evaluate the pH effects. (Overall treatment mean; Max. value = 4.32, Min. value = 4.25); LSD value days = 0.0063, LSD value treatments = 0.0118, LSD value interactions (days x treatments) = 0.0264; Control: (T_0, T_0'; without guar gum), CGG: Crude guar gum; (T_1, 0.1%; T_2, 0.5%; T_3, 1%); PGG: Purified guar gum; (T_4, 0.1%; T_5, 0.5%; T_6, 1%), AHGG: Acid hydrolyzed guar gum; (T_7, 0.1%; T_8, 0.5%; T_9, 1%), BHGG: Base hydrolyzed guar gum; (T_{10}, 0.1%; T_{11}, 0.5%; T_{12}, 1%), EHGG: Enzyme hydrolyzed guar gum; (T_{13}, 0.1%; T_{14}, 0.5%; T_{15}, 1%).

The results indicated that controlled, as well as treated samples, exhibited a decreasing trend in WHC. It is apparent from the results that AHGG (1%) and EHGG (0.5%) showed less WHC comparatively. Lower WHC is related to unstable and excessive rearrangements of a weak network of gel. The acid and enzyme hydrolyzed guar gum are suitable for yoghurt development as these guar gum have less viscosity [6,45]. The highest mean value of WHC observed was 83.1% in T_9 (1% AHGG) at 0 days of storage, whereas the lowest WHC (27.2%) was found in T_{12} (1% BHGG) on the 28th day of storage as far as interaction among the treatments and storage days is concerned. The findings of the current study are supported by Bahrami and Ahmadi [55] who evaluated that syneresis and WHC in the yoghurt samples were influenced by the kind and level of stabilizer. WHC in samples containing 0.1% of guar gum had significant variation ($p < 0.05$) compared to the control sample. With an increased concentration of guar gum, there was an incremental reduction in WHC, so the minimum WHC perceived in the sample containing guar gum was 0.3%.

3.9. Textural Analysis of the Prepared Yoghurt with Hydrolyzed and Non-Hydrolyzed Guar Gum

Data regarding firmness revealed that the storage interval affected the parameter significantly as it increased with the storage period. The maximum firmness was observed at 0.9371 N on the 28th day and the minimum was 0.8047 N at 0 days of storage. This situation could be attributed to the increased water-holding capacity of milk proteins with time storage. The controlled samples showed lower values for firmness. Akalın and Unal [56] and Ekinci and Gurel [57] reported an increase in firmness with the storage period while studying the changes in the functional properties of yoghurt. The consistency of yoghurt was affected significantly as it decreased throughout the storage period from 0 days to 28th days. The consistency had the highest mean value of 47.0 N at 0 days and the lowest mean value of 21.2 N on the 28th day of storage. All the treatments showed a decrease in consistency during storage which might have been due to increased syneresis with the passage of time. The controlled samples (without guar gum) indicated that consistency in T_0 and $T_{0'}$ was 51.6 N and 59.0 N, respectively. The results of the current study are in agreement with the findings of Yadav and Jain [58]. They found that there was a decrease in the consistency of yoghurt with the passage of time while studying changes during storage of probiotic Dahi (fermented milk product originating from India) at 7 °C.

Cohesiveness showed that storage had a significant effect on it as it increased throughout the storage period from 0 to 28th days. The cohesiveness had the highest mean value of −0.52 on the 28th day and the lowest mean value of −0.34 at 0 days of storage. The controlled samples (without guar gum) indicated that cohesiveness in T_0 and $T_{0'}$ was −0.3. Seckin and Ozkilinc [59] found that the storage period had a significant impact on the cohesiveness of prebiotics strained yoghurt. Cohesiveness values were increased during storage. Data regarding adhesiveness showed that storage had a significant impact on adhesiveness as it increased throughout the storage period from 0 to 28th days. The adhesiveness had the highest mean value of 3.38 on the 28th day and the lowest mean value of 2.58 at 0 days of storage. The controlled samples (without guar gum) indicated that adhesiveness in T_0 and $T_{0'}$ was 3.0 and 3.2 N, respectively. The controlled samples showed higher values for adhesiveness. The results of the current study are in line with the findings of Fadela and Abderrahim [60] and Seckin and Ozkilinc [59]. Gustaw and Kordowska-Wiater [61] while studying the influence of prebiotics on the growth of lactic acid bacteria reported that there was a steady increase in adhesiveness with the dose of prebiotics incorporated and with the passage of time. The results showed that crude, purified and basic hydrolyzed guar gum proved ineffective when used at higher levels i.e., T_2 (0.5% CGG), T_3 (1% CGG), T_5 (0.5% PGG), T_6 (1% PGG), T_{11} (0.5% BGG) and T_{12} (1% BGG). This was observed due to the phase separation of casein–guar mixtures because of the higher concentration of guar gum. The results obtained in this study are in line with those of Gustaw and Kordowska-Wiater [61] who concluded that at low guar gum concentrations a denser network was formed, whereas higher guar gum concentrations led

to phase separation (filamentous or protein-rich droplets) during their work on designing microstructure into acid skim milk/guar gum gels.

4. Conclusions

CGG was passed through hydrolysis procedures to develop various hydrolyzed derivatives. The prebiotic potential of PHGG was determined to improve its bioavailability. The microstructural evaluation revealed that hydrolyzed guar gum derivatives produced by enzymatic action provided better results as compared to others. The SEM micrographs and XRD pattern of EHGG depicted well defined porous structure with an excellent interconnected framework and reduced compactness with a bit higher crystallinity index, developed by the mannanase enzyme. FTIR spectroscopy showed no major change in the structure of hydrolyzed derivatives. PHGG has possessed prebiotic properties to support the growth of *B. bifidum*. The hydrolyzed (acidic and enzymatically) guar gum with the level of 0.5% and 1% offered the best results for the physicochemical and textural parameters of set-type yoghurt. Conclusively, it can be declared that hydrolyzed derivatives of guar gum have good thickening, emulsifying and gelling properties with increased utility in food applications.

Author Contributions: Conceptualization, M.H.; methodology, S.A.; software, N.K., M.W.I. and T.M.; validation, T.M. and M.A.; formal analysis, N.K., M.W.I. and N.W.; investigation, M.H., T.M., S.A.K., T.E. and S.A.; resources, T.I., I.M.K., S.A.K. and T.E.; data curation, N.K., M.W.I., S.A.K., T.E. and N.W.; writing—original draft, M.H. and S.A.; writing—review & editing, M.A., T.I., M.W.I., N.W., S.A.K., T.E. and T.M. visualization, M.A., I.M.K., S.A.K. and T.E.; supervision, M.A.; project administration, M.H., M.A., T.I., S.A.K. and T.E.; funding acquisition, M.H., M.A., S.A.K. and T.E. All authors have read and agreed to the published version of the manuscript.

Funding: The publication of this article was supported by the Open Access Fund of Leibniz Universität Hannover. This study received no external fundings.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors are thankful to the University of Agriculture, Faisalabad; Bahauddin Zakariya University, Multan-Pakistan and Higher Education Commission, Pakistan for providing facilities for the research work.

Conflicts of Interest: Authors declare no conflict of interest.

References

1. McRorie, J.W., Jr. The physics of fiber in the gastrointestinal tract: Laxation, antidiarrheal, and irritable bowel syndrome. In *Dietary Interventions in Gastrointestinal Diseases*; Elsevier: San Diego, CA, USA, 2019; pp. 19–32.
2. Niv, E.; Halak, A.; Tiommny, E.; Yanai, H.; Strul, H.; Naftali, T.; Vaisman, N. Randomized clinical study: Partially hydrolyzed guar gum (PHGG) versus placebo in the treatment of patients with irritable bowel syndrome. *Nutr. Metab.* **2016**, *13*, 10. [CrossRef] [PubMed]
3. Ho, C.Y.; Ahmad, A.F.; Woo, S.S.; Selvarajoo, T.; Jamhuri, N.; Kahairudin, Z. Integrate partial hydrolyzed guar gum in postoperative ileostomy nutritional management. *J. Med. Res. Innov.* **2020**, *4*, e000206. [CrossRef]
4. Mudgil, D. Partially hydrolyzed guar gum: Preparation and properties. In *Polymers for Food Applications*; Springer: Berlin/Heidelberg, Germany, 2018; pp. 529–549.
5. Singh, V.; Tiwari, A. Hydrolytic fragmentation of seed gums under microwave irradiation. *Int. J. Biol. Macromol.* **2009**, *44*, 186–189. [CrossRef] [PubMed]
6. Yoon, S.-J.; Chu, D.-C.; Raj Juneja, L. Chemical and physical properties, safety and application of partially hydrolized guar gum as dietary fiber. *J. Clin. Biochem. Nutr.* **2008**, *42*, 1–7. [CrossRef] [PubMed]
7. Hussain, M.; Zahoor, T.; Akhtar, S.; Ismail, A.; Hameed, A. Thermal stability and haemolytic effects of depolymerized guar gum derivatives. *J. Food Sci. Technol.* **2018**, *55*, 1047–1055. [CrossRef]

8. Reider, S.J.; Moosmang, S.; Tragust, J.; Trgovec-Greif, L.; Tragust, S.; Perschy, L.; Przysiecki, N.; Sturm, S.; Tilg, H.; Stuppner, H. Prebiotic effects of partially hydrolyzed guar gum on the composition and function of the human microbiota—Results from the PAGODA Trial. *Nutrients* **2020**, *12*, 1257. [CrossRef]
9. El Fray, M.; Pilaszkiewicz, A.; Swieszkowski, W.; Kurzydlowski, K.J. Morphology assessment of chemically modified cryostructured poly (vinyl alcohol) hydrogel. *Eur. Polym. J.* **2007**, *43*, 2035–2040. [CrossRef]
10. Birkholz, M. *Thin Film Analysis by X-ray Scattering*; Wiley-VCH Verlag GmbH & Co., KGaA: Weinheim, Germany, 2006.
11. Afzaal, M.; Saeed, F.; Saeed, M.; Azam, M.; Hussain, S.; Mohamed, A.A.; Alamri, M.S.; Anjum, F.M. Survival and stability of free and encapsulated probiotic bacteria under simulated gastrointestinal and thermal conditions. *Int. J. Food Prop.* **2020**, *23*, 1899–1912. [CrossRef]
12. Azam, M.; Saeed, M.; Yasmin, I.; Afzaal, M.; Ahmed, S.; Khan, W.A.; Iqbal, M.W.; Hussain, H.T.; Asif, M. Characterization, Microencapsulation and invitro characterization of Bifidobacterium animalis for improved survival. *J. Food Meas. Charact.* **2021**, *15*, 2591–2600. [CrossRef]
13. Fukui, H.; Oshima, T.; Tanaka, Y.; Oikawa, Y.; Makizaki, Y.; Ohno, H.; Tomita, T.; Watari, J.; Miwa, H. Effect of probiotic Bifidobacterium bifidum G9-1 on the relationship between gut microbiota profile and stress sensitivity in maternally separated rats. *Sci. Rep.* **2018**, *8*, 12384. [CrossRef]
14. Lim, H.J.; Shin, H.S. Antimicrobial and immunomodulatory effects of bifidobacterium strains: A review. *J. Microbiol. Biotechnol.* **2020**, *30*, 1793–1800. [CrossRef]
15. Azam, M.; Saeed, M.; Ahmad, T.; Yamin, I.; Khan, W.A.; Iqbal, M.W.; Mahmood, S.; Rizwan, M.; Riaz, T. Characterization, Characterization of biopolymeric encapsulation system for improved survival of Lactobacillus brevis. *J. Food Meas. Charact.* **2022**, *16*, 2292–2299. [CrossRef]
16. Ofori-Kwakye, K.; Asantewaa, Y.; Kipo, S.L. Physicochemical and binding properties of cashew tree gum in metronidazole tablet formulations. *Int. J. Pharm. Pharm. Sci.* **2010**, *2*, 105–109.
17. Chauhan, K.; Chauhan, G.S.; Ahn, J.-H. Synthesis and characterization of novel guar gum hydrogels and their use as Cu^{2+} sorbents. *Bioresour. Technol.* **2009**, *100*, 3599–3603. [CrossRef]
18. Beltrán, O.; de Pinto, G.L.; Rincón, F.; Picton, L.; Cozic, C.; Le Cerf, D.; Muller, G. Acacia macracantha gum as a possible source of arabinogalactan–protein. *Carbohydr. Polym.* **2008**, *72*, 88–94. [CrossRef]
19. Cheng, Y.; Prud'homme, R.K. Enzymatic degradation of guar and substituted guar galactomannans. *Biomacromol.* **2000**, *1*, 782–788. [CrossRef]
20. Sen, G.; Mishra, S.; Jha, U.; Pal, S. Microwave initiated synthesis of polyacrylamide grafted guar gum (GG-g-PAM)—Characterizations and application as matrix for controlled release of 5-amino salicylic acid. *Int. J. Biol. Macromol.* **2010**, *47*, 164–170. [CrossRef]
21. Mudgil, D.; Barak, S.; Khatkar, B. X-ray diffraction, IR spectroscopy and thermal characterization of partially hydrolyzed guar gum. *Int. J. Biol. Macromol.* **2012**, *50*, 1035–1039. [CrossRef]
22. Gupta, S.; Shah, B.; Sanyal, B.; Variyar, P.S.; Sharma, A. Role of initial apparent viscosity and moisture content on post irradiation rheological properties of guar gum. *Food Hydrocoll.* **2009**, *23*, 1785–1791. [CrossRef]
23. Đurđević-Denin, J.D.; Maćej, O.D.; Jovanović, S.T. Viscosity of set-style yogurt as influenced by heat treatment of milk and added demineralized whey powder. *J. Agric. Sci.* **2002**, *47*, 45–56.
24. Hasan, M.; Huma, N.; Sameen, A.; Rafiq, S.; Gulzar, N. Use of meteroxylon sagu as a stabilizing agent in yoghurt. *J. Food Chem. Nutr.* **2014**, *2*, 19–26.
25. Wacher-Rodarte, C.; Galvan, M.V.; Farres, A.; Gallardo, F.; Marshall, V.M.; Garcia-Garibay, M. Yogurt production from reconstituted skim milk powders using different polymer and non-polymer forming starter cultures. *J. Dairy Res.* **1993**, *60*, 247–254. [CrossRef]
26. Singh, G.; Muthukumarappan, K. Influence of calcium fortification on sensory, physical and rheological characteristics of fruit yogurt. *LWT-Food Sci. Technol.* **2008**, *41*, 1145–1152. [CrossRef]
27. Brennan, C.S.; Tudorica, C.M. Carbohydrate-based fat replacers in the modification of the rheological, textural and sensory quality of yoghurt: Comparative study of the utilisation of barley beta-glucan, guar gum and inulin. *Int. J. Food Sci. Tech.* **2008**, *43*, 824–833. [CrossRef]
28. AOAC. *Official Methods of Analysis of AOAC International*, 17th ed.; AOAC International: Gaithersburg, MD, USA, 2000.
29. Hara, H.; Suzuki, T.; Kasai, T.; Aoyama, Y.; Ohta, A. Ingestion of guar gum hydrolysate, a soluble fiber, increases calcium absorption in totally gastrectomized rats. *J. Nutr.* **1999**, *129*, 39–45. [CrossRef]
30. Scholz-Ahrens, K.E.; Ade, P.; Marten, B.; Weber, P.; Timm, W.; Açil, Y.; Glüer, C.-C.; Schrezenmeir, J. Prebiotics, probiotics, and synbiotics affect mineral absorption, bone mineral content, and bone structure. *J. Nutr.* **2007**, *137*, 838S–846S. [CrossRef]
31. Wang, L.-F.; Pan, S.-Y.; Hu, H.; Miao, W.-H.; Xu, X.-Y. Synthesis and properties of carboxymethyl kudzu root starch. *Carbohydr. Polym.* **2010**, *80*, 174–179. [CrossRef]
32. Zheng, J.; Cui, Z.P.; Li, Z.B.; Gao, Y.H.; Zhang, J.X. In preparation and characteristics of guar gum-sodium alginate complexed bone adhesive material. In Proceeding of the International Seminar on Future Biomedical Information Engineering, Wuhan, China, 18 December 2008; pp. 353–356.

33. Wang, Q.; Ellis, P.R.; Ross-Murphy, S.B. Dissolution kinetics of guar gum powders—III. Effect of particle size. *Carbohydr. Polym.* **2006**, *64*, 239–246. [CrossRef]
34. Cunha, P.L.; Castro, R.R.; Rocha, F.A.; de Paula, R.C.; Feitosa, J.P. Low viscosity hydrogel of guar gum: Preparation and physicochemical characterization. *Int. J. Biol. Macromol.* **2005**, *37*, 99–104. [CrossRef]
35. Mudgil, D.; Barak, S.; Khatkar, B.S. Effect of enzymatic depolymerization on physicochemical and rheological properties of guar gum. *Carbohydr. Polym.* **2012**, *90*, 224–228. [CrossRef]
36. Prasad, S.S.; Rao, K.M.; Reddy, P.R.S.; Reddy, N.S.; Rao, K.K.; Subha, M. Synthesis and characterisation of guar gum-g-poly (acrylamidoglycolic acid) by redox initiator. *Ind. J. Adv. Chem. Sci.* **2012**, *1*, 28–32.
37. López-Franco, Y.; Cervantes-Montaño, C.; Martínez-Robinson, K.; Lizardi-Mendoza, J.; Robles-Ozuna, L. Physicochemical characterization and functional properties of galactomannans from mesquite seeds (*Prosopis* spp.). *Food Hydrocoll.* **2013**, *30*, 656–660. [CrossRef]
38. Sun, J.; Sun, X.; Sun, R.; Su, Y. Fractional extraction and structural characterization of sugarcane bagasse hemicelluloses. *Carbohydr. Polym.* **2004**, *56*, 195–204. [CrossRef]
39. Shobha, M.; Vishukumar, A.; Tharanathan, R.; Koka, R.; Gaonkar, A. Modification of guar galactomannan with the aid of pectinase. *Carbohydr. Polym.* **2005**, *62*, 267–273. [CrossRef]
40. Gong, H.; Liu, M.; Chen, J.; Han, F.; Gao, C.; Zhang, B. Synthesis and characterization of carboxymethyl guar gum and rheological properties of its solutions. *Carbohydr. Polym.* **2012**, *88*, 1015–1022. [CrossRef]
41. Dodi, G.; Hritcu, D.; Popa, M. Carboxymethylation of guar gum: Synthesis and characterization. *Cellul. Chem. Technol.* **2011**, *45*, 171–176.
42. Kačuráková, M.; Wilson, R. Developments in mid-infrared FT-IR spectroscopy of selected carbohydrates. *Carbohydr. Polym.* **2001**, *44*, 291–303. [CrossRef]
43. Kacurakova, M.; Capek, P.; Sasinkova, V.; Wellner, N.; Ebringerova, A. FT-IR study of plant cell wall model compounds: Pectic polysaccharides and hemicelluloses. *Carbohydr. Polym.* **2000**, *43*, 195–203. [CrossRef]
44. Wang, J.; Somasundaran, P. Study of galactomannose interaction with solids using AFM, IR and allied techniques. *J. Colloid Interface Sci.* **2007**, *309*, 373–383. [CrossRef]
45. Mudgil, D.; Barak, S.; Khatkar, B.S. Guar gum: Processing, properties and food applications—A Review. *J. Food Sci. Technol.* **2014**, *51*, 409–418. [CrossRef]
46. Cruz, A.; Cavalcanti, R.; Guerreiro, L.; Sant'Ana, A.; Nogueira, L.; Oliveira, C.; Deliza, R.; Cunha, R.; Faria, J.; Bolini, H. Developing a prebiotic yogurt: Rheological, physico-chemical and microbiological aspects and adequacy of survival analysis methodology. *J. Food Eng.* **2013**, *114*, 323–330. [CrossRef]
47. Mazloomi, S.; Shekarforoush, S.; Ebrahimnejad, H.; Sajedianfard, J. Effect of adding inulin on microbial and physicochemical properties of low fat probiotic yogurt. *Iran. J. Vet. Res.* **2011**, *12*, 93–98.
48. Prasanna, P.; Grandison, A.; Charalampopoulos, D. Microbiological, chemical and rheological properties of low fat set yoghurt produced with exopolysaccharide (EPS) producing Bifidobacterium strains. *Food Res. Int.* **2013**, *51*, 15–22. [CrossRef]
49. Shaghaghi, M.; Pourahmad, R.; Adeli, H.M. Synbiotic yogurt production by using prebiotic compounds and probiotic lactobacilli. *J. Basic Appl. Sci.* **2013**, *5*, 839–846.
50. Khalifa, M.; Elgasim, A.; Zaghloul, A.; Mahfouz, M. Applications of inulin and mucilage as stabilizers in yoghurt production. *Am. J. Food Technol.* **2011**, *6*, 31–39. [CrossRef]
51. Fadela, C.; Abderrahim, C.; Ahmed, B. Use of lactic strains isolated from Algerian ewe's milk in the manufacture of a natural yogurt. *Afr. J. Biotechnol.* **2008**, *7*, 1181–1186.
52. Ahmad, S.; Gaucher, I.; Rousseau, F.; Beaucher, E.; Piot, M.; Grongnet, J.F.; Gaucheron, F. Effects of acidification on physico-chemical characteristics of buffalo milk: A comparison with cow's milk. *Food Chem.* **2008**, *106*, 11–17. [CrossRef]
53. Karaca, O.B. Effects of different prebiotic stabilisers and types of molasses on physicochemical, sensory, colour and mineral characteristics of probiotic set yoghurt. *Int. J. Dairy Technol.* **2013**, *66*, 490–497. [CrossRef]
54. Hematyar, N.; Samarin, A.M.; Poorazarang, H.; Elhamirad, A.H. Effect of gums on yogurt characteristics. *World Appl. Sci. J.* **2012**, *20*, 661–665.
55. Bahrami, M.; Ahmadi, D.; Alizadeh, M.; Hosseini, F. Physicochemical and sensorial properties of probiotic yogurt as affected by additions of different types of hydrocolloid. *Food Sci. Anim. Resour.* **2013**, *33*, 363–368. [CrossRef]
56. Akalın, A.; Unal, G.; Dinkci, N.; Hayaloglu, A. Microstructural, textural, and sensory characteristics of probiotic yogurts fortified with sodium calcium caseinate or whey protein concentrate. *J. Dairy Sci.* **2012**, *95*, 3617–3628. [CrossRef]
57. Ekinci, F.; Gurel, M. Effect of using propionic acid bacteria as an adjunct culture in yogurt production. *J. Dairy Sci.* **2008**, *91*, 892–899. [CrossRef]
58. Yadav, H.; Jain, S.; Sinha, P. Evaluation of changes during storage of probiotic Dahi at 7 C. *Int. J. Dairy Technol.* **2007**, *60*, 205–210. [CrossRef]
59. Seckin, A.K.; Ozkilinc, A.Y. Effect of some prebiotics usage on quality properties of concentrated yogurt. *J. Anim. Vet. Adv.* **2011**, *10*, 1117–1123. [CrossRef]

60. Fadela, C.; Abderrahim, C.; Ahmed, B. Sensorial and physicochemical characteristics of yoghurt manufactured with ewe's and skim milk. *World J. Dairy Food Sci.* **2009**, *4*, 136–140.
61. Gustaw, W.; Kordowska-Wiater, M.; Kozioł, J. The influence of selected prebiotics on the growth of lactic acid bacteria for bio-yoghurt production. *Acta Sci. Pol. Technol. Aliment.* **2011**, *10*, 455–466.