

Impact of Inulin Extracted, Purified from (Chicory and Globe Artichoke) Roots and the Combination with Maltodextrin as Prebiotic Dietary Fiber on the Functional Properties of Stirred Bio-Yogurt

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Abstract

Inulin is a prebiotic dietary fiber that plays an integral role in producing functional dairy products with improved health benefits. Therefore, the objectives of this study are as follows: extract and purify inulin from chicory roots and globe artichoke roots; evaluate the physicochemical, functional properties and functional groups of the purified inulin; determine the functional properties of chicory roots inulin-maltodextrin and globe artichoke roots inulin-maltodextrin and compare it with that of the commercial inulin; examine the impact of various inulin on physicochemical, microstructural, textural, sensory characteristics and as prebiotic dietary fiber on probiotic bacteria's viability of stirred bio-yogurt. The characteristics of the microstructure were investigated by scanning electron microscopy and, Fourier transforms infrared spectroscopy to detect the functional group. The resulting inulin exhibited a high yield and purity along with enhanced functional properties. Stirred bio-yogurt fortified with chicory roots inulin or globe artichoke roots inulin showed enhanced physicochemical, microstructural, microbiological, and overall sensorial acceptability followed by chicory roots inulin-maltodextrin or globe artichoke roots inulin-maltodextrin and the commercial inulin as compared to the control. Stirred bio-yogurt samples can offer various health benefits and wide applications as supplement of prebiotic dietary fiber in dairy industry.

Keywords

Inulin, Chicory Roots, Globe Artichoke Roots, Prebiotic Dietary Fibers, Stirred Bio-Yogurt

1. Introduction

Inulin is present in many plant species such as chicory root (*Cichorium intybus* L.) and globe artichoke root (*Cynara scolymus* L.) two commercial sources of inulin production. Inulin, a non-digestible polysaccharide containing naturally occurring fructo-oligosaccharides, is a linear fructose polymer with beta glycosidic linkages β -(2 \rightarrow 1) D-fructosyl-fructose bonds [1]. Inulin can be used to improve some functional properties such as texture modification [2]. It can also be used as a gelling agent, as a bulking agent in reduced sugar applications, in emulsification, as a replacement of sugar (a low-calorie sweetener due to its non-digestibility in the human alimentary system and contributes to 25% - 30% of energy and 10% of the sweetness of sucrose, fat replacer and wall material [3].

Recent years have seen, an increasing interest in the consumption and production of dairy products that exert a positive effect on health beyond their nutritional value. Among these functional dairy products, much attention has been focused on probiotic products and food-containing dietary fiber. One of the approaches to increase the number of probiotic bacteria in the intestinal microbiota is the inclusion of prebiotics in the food systems, which are non-digestible dietary fiber components, mainly including carbohydrates such as inulin [4].

Dairy products are not a good source of dietary fiber; however, they can provide an alternative for the development of dietary fiber enriched dairy products by fortification with inulin. Adding inulin into yogurt not only rheological attributes, but also exhibits little sensory properties and increases the shelf-life of dairy products that have improved nutritional and health benefits along with certain industrial properties [3]. It is not broken down by the gut digestive enzymes and absorption in the small intestine; however, it is fermentable by the microflora of large intestines, further producing short chain fatty acids that lower the interstitial pH. This process causes the selective stimulated growth of bifidobacterial population in the large intestines, is effective against some colon disorders, such as constipation regulates blood glucose levels for diabetes management, reduces LDL cholesterol and serum lipid levels, enhances calcium absorption, and positively affects the immune system [5].

The combination of maltodextrin and inulin is widely used for producing dairy products with better properties. Maltodextrin is a polysaccharide that comprises D-glucose monomers linked by the glycoside bond α (1 \rightarrow 4), which is obtained from the partial hydrolysis of starch. Maltodextrin can be used to improve some functional properties such as flavor enhancers, fat replacers and bulking agents in dairy product. Several researchers have described the effects of fat replacer such as inulin and maltodextrin on the sensory quality of low-fat fermented milk [6].

Therefore, has multiple objectives of this study: extract and purify inulin from chicory roots and globe artichoke roots; evaluate the physicochemical, functional properties and functional groups of the purified inulin; determine the functional properties of chicory roots inulin-maltodextrin and globe artichoke roots in-

ulin-maltodextrin and compare it with that of the commercial inulin; examine the impact of various inulin on physiochemical, microstructural, textural, sensory characteristics and as prebiotic dietary fiber on probiotic bacteria's viability of stirred bio-yogurt.

2. Material and Methods

2.1. Materials

Chicory roots (CR) were obtained from the local fields of the agriculture farm of the Alexandria University, Egypt, and globe artichoke roots (GAR) were obtained from Kafr El-Dawar, El Beheira Governorate, Egypt. Fresh buffalo milk was obtained from the Faculty of Agriculture farm of the Alexandria University, Egypt. Skimmed milk powder comprising 34% protein, 51% lactose, 1.2% fat, 8.2% minerals, and 4% moisture was obtained from Dairy America, Inc., California, USA. Commercial inulin was obtained from food additives company and maltodextrin (M) was obtained from China. Standard inulin was obtained from Sigma Aldrich, Merck (St. Louis, MO, USA). Commercially freeze-dried lactic acid starter cultures for a direct-to-vat set, YF-L903 containing *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (1:1), and probiotic starter culture (ABT-5) comprising (*Streptococcus thermophilus* ST-20Y, *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12) were used in the study. Both starter cultures were obtained from Christen Hansen Laboratories, Copenhagen, Denmark, and kept at -18°C . After preliminary incubation, ABT-5 was added to the milk for 18 h at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

2.2. Methods

2.2.1. Preparation of Raw Material

CR and GAR were carefully washed with tap water to remove the remaining soil and other undesirable materials, then peeled and cut into slices.

2.2.2. Extraction of Inulin

Inulin was extracted from CR and GAR following the method described by [7].

2.2.3. Determination of Inulin Yield

The yield of inulin (%) was calculated according to the following formula

$$\text{Yield}(\%) = \frac{\text{Yield of inulin (Kg)}}{\text{Yield of dry weight taken for extraction (Kg)}} \times 100$$

2.2.4. Characterization of Inulin

1) Physicochemical properties

Appearance, taste, flavor, sweetness, and solubility of inulin were assessed according to [8]. The pH was measured using a digital pH meter (Persica pH 900, Switzerland). Dry matter, fat, protein, ash, sodium, vitamins, minerals, dietary fiber content and reducing sugars were determined in accordance with [9]. Caloric value was calculated ($\text{Protein} \times 4 + \text{reducing sugar} \times 4 + \text{fat} \times 9 + \text{dietary}$

fiber $\times 2$) Kcal/100 g [10]. The color was determined by using a Hunter colorimeter (Hunter Ultra Scan VIS).

2) *Functional properties*

Functional properties of chicory roots inulin (CRI), globe artichoke roots inulin (GARI), chicory roots inulin-maltodextrin (CRIM) at a ratio (1:2 w/w) respectively, globe artichoke roots inulin-maltodextrin (GARIM) at a ratio (1:2 w/w) respectively and commercial inulin. The water-holding capacity (WHC) and oil-holding capacity (OHC) were determined following the method [11] and results were expressed either as g of water or sunflower oil per g of sample. The emulsifying activity (EA) was described by [12] and values were calculated as follows:

$$EA\% = \text{Volume of the emulsion phase} / \text{Total volume of the system} \times 100.$$

The swelling power (SP) was described by [13].

3) *Functional groups*

Functional groups of inulin samples were acquired using a Fourier transform infrared spectroscopy (FTIR) spectrophotometer (Shimadzu FTIR-8400 S, Japan) that was equipped with an ATR 8000A accessory. The spectra were acquired over the range of 4000 - 400 cm^{-1} [14].

2.2.5. Stirred Bio-Yogurt (SBY) Preparation

Fresh buffalo's milk was skimmed and standardized to 0.5% fat and 14% total solids by adding 3% Skimmed milk powder, and the milk was divided into six equivalent batches as follows: C: Control plain SBY without addition; T₁: SBY fortified with 2% commercial inulin; T₂: SBY fortified with 2% CRI; T₃: SBY fortified with 2% CRIM; T₄: SBY fortified with 2% GARI; and T₅: SBY fortified with 2% GARIM. All the treatments were separately homogenized at 2500 psi with an Ultra-Turrax blender (IKA, Merck, Germany) at 14,000 rpm until all the ingredients were dissolved in the milk. The milk was heated separately at 80°C for 15 min and then cooled to 42°C \pm 1°C. Then, all the batches were inoculated with the yogurt starter culture (0.03/Kg w/w) and the ABT-5 probiotic starter culture (0.05/Kg w/w) and incubated at 42°C \pm 1°C until a firm curd was obtained. The curd was then refrigerated at 4°C overnight before being stirred, packaged and immediately transferred to a refrigerator at 4°C \pm 1°C for storing up to 21 days [15]. The SBY samples were analyzed for their physicochemical, microbiological, microstructural, and sensory properties on the first day after preparation and during the cold storage.

2.2.6. Characteristics of SBY

1) *Physiochemical analysis*

The total solid and titratable acidity (% lactic acid) were determined by [9]. The pH was measured using a digital pH meter (Persica pH 900, Switzerland). Synaeresis was determined using [16]. The color analyses were conducted for the fresh samples, using a Hunter colorimeter (Hunter Ultra Scan VIS). The apparent viscosity was measured at fresh and after 21 days of cold storage, using a

Bohlin coaxial cylinder viscometer (Bohlin Instrument Inc., Sweden), which was attached to a workstation loaded with the V88 viscometer programming software. The viscometer probe, system C30, was placed in the SBY sample cup, and the measurements of viscosity were conducted at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ in the up mode at shear rates ranging from 19 to 126 s^{-1} .

2) Microstructural characterization

The microstructures of SBY samples were analyzed using a scanning electron microscope (Joel Jsm 6360LA, Japan) after vacuum coating the surfaces with gold [17]. For preparation of SBY samples, cubes ($3 \pm 0.5\text{ mm}^3$) were cut from different areas of SBY cup and fixed in 3% glutaraldehyde in 0.05 M phosphate buffer (pH 7) at 4°C for 4 h. The fixed cubes were dehydrated by consecutive soaking in 30%, 50%, 70% and 95% ethanol each for 20 min, and finally was rinsed successively twice by absolute ethanol (100%) at 48°C and 58°C , respectively. Cubes were immediately dried in the critical point drier (Samdri PVT-3B, Tousimis, Rockville, MD) for 5 h using CO_2 . The dried cube was fractured and mounted on sputter. The SBY samples were viewed at magnification of 1000 and 5000. The analysis was carried out after 7 days of preparation of SBY samples.

3) Microbiological analysis:

The enumeration of *Streptococcus thermophilus* counts was performed on the M17 agar (Biolife, Italy) and incubated at 37°C for 48 h. under aerobic conditions. *Lactobacillus bulgaricus* counts were enumerated using the MRS agar (Biolife, Italy) and incubated aerobically at 37°C for 72 h [18]. *Lactobacillus acidophilus* counts were determined using the MRS-bile agar for this purpose, and the MRS agar was prepared with 1.5 g/L of bile agar. Alternatively, the selective enumeration of *Bifidobacterium bifidum* was performed on the MRS-cysteine agar that was prepared with 0.05 g/L of cysteine. Conversely, the plates of *Bifidobacterium bifidum* and *Lactobacillus acidophilus* cultures were incubated at 37°C for 72 h under anaerobic conditions using a gas pack (Oxide, UK). The molds and yeasts were enumerated by [19] guidelines, using potato dextrose agar (Difco, Italy) acidified with 10% tartaric acid and incubated at 25°C for 5 days. Violet red bile lactose agar (Oxoid; England) was used to count the coliform [20]. The plates were incubated at 37°C for 24 h. The colony-forming units were measured as $\log\text{ cfu g}^{-1}$.

4) Sensory evaluation

The sensory evaluation of the SBY samples was assessed by 20 panellists (11 men and 9 women aged 24 - 55 years) at the Dairy Research Department, Food Technology Research Institute (FTRI), Agricultural Research Centre. The selected panel consisted as defined by [21]. The criteria for selection depended on their experience and background related to SBY samples. The samples, which were stored at 4°C , were allowed to rest at room temperature (25°C), 10 min before evaluation. The samples were evaluated using a nine-point hedonic scale [22].

2.3. Statistical Analysis

Data were expressed as means \pm standard deviations values of the triplicate sam-

ples, which were analyzed by using multiple comparisons, one-way analysis of variance (ANOVA), and the Duncan test. Statistically significant results of physicochemical and descriptive sensory evaluations of SBY samples were analyzed by two-way analysis of variance ANOVA followed by a t-test (LSD) using SAS version 9.1 (SAS Institute Inc.). The significance levels of $P \leq 0.05$ were used for statistical differences, and the least significant difference (LSD) was calculated for the comparison between means.

3. Results and Discussion

3.1. Yield Parameters of CRI and GARI

Data presented in **Table 1** show the obtained yield of fresh, dry weight, and inulin varieties extracted from CR and GAR. Furthermore, data in this table indicate that such variety produced a high yield of fresh and dry weight as well as inulin yield in two sources of inulin. The yield of inulin (w/w) extracted from GAR (24%) was significantly ($P \leq 0.05$) higher than that of CR (14.7%). These results are in line with [23].

3.2. Characterization of CRI and GARI

3.2.1. Physicochemical Properties

Data presented in **Table 1**, show the physicochemical properties of CRI, GARI and commercial inulin. CRI, GARI and commercial inulin, exhibited the same physicochemical properties. CRI and GARI were white or white to grey powder as shown by a higher L^* value and slightly higher a^* and b^* values with fine particles having a greater clarity. It had a neutral taste, without off-flavor or after-taste and less sweet than sugar (10% sweetness of the sucrose). Inulin was moderately soluble in water (nearly 100 mg/ml at 25°C) and colorless (clear to hazy). The pH was 5.5 - 7.0. Our study results agreed with [8]. It was obviously clear that the CRI and GARI had low caloric values of 194 and 193.2 kcal/100g, respectively, and a high concentration of dietary fiber of 88 g/100g. Moreover, inulin had negligible amount of fat, protein, ash, vitamins, and minerals.

3.2.2. Functional Properties

Figure 1(a) and **Figure 1(b)** shows the comparison of the functional properties of CRI, CRIM, GARI and GARIM with the functional properties of commercial inulin.

1) *Water-holding capacity (WHC):*

The WHC of CRI and GARI had the highest values (0.68 and 0.70 g water/g sample, respectively) as compared with CRIM and GARIM (0.30 and 0.32 g water/g sample, respectively) due to maltodextrin is easily dissolved in water with low hygroscopicity and high-water solubility and form sticky solutions. Furthermore, the results indicated that the addition of maltodextrin in CRI and GARI show the same results of the WHC combination is compared with that of the commercial inulin (0.31 g water/g sample). High WHC is directly related with the presence of hydrophilic regions in the structure [24]. Hydroxyl groups

Table 1. Yield parameters of inulin and physicochemical properties of Chicory roots inulin, *Globe artichoke* roots inulin and *Commercial inulin*.

	Chicory roots	<i>Globe artichoke</i> roots	<i>Commercial inulin</i>
<u>Yield parameters</u>			
Yield of fresh (Kg)	100 ± 0.00 ^a	100 ± 0.00 ^a	
Yield of dry weight (Kg)	17 ± 1.00 ^a	15 ± 1.00 ^a	
Yield of inulin (Kg)	2.5 ± 0.17 ^b	3.6 ± 0.10 ^a	
The yield of inulin (%)	14.7 ± 0.2 ^b	24 ± 0.3 ^a	
<u>Attributes</u>			
Appearance			
(Color)	White to grey	White	White
(Form)	Powder	Powder	Powder
Taste	Neutral	Neutral	Neutral
Flavor	No off flavor	No off flavor	No off flavor
Sweetness (v. sucrose = 100)	Less sweet than sugar (10% of the sucrose)	Less sweet than sugar (10% of the sucrose)	Less sweet than sugar (10% of the sucrose)
Solubility 100 mg/ml at 25°C	Moderately	Moderately	Moderately
(Color)	Colorless	Colorless	Colorless
(Turbidity)	Clear to Hazy	Clear to Hazy	Clear to Hazy
pH value (10% w/w)	5.5 - 7.0	5.5 - 7.0	5 - 7
<u>Chemical properties (g/100g)</u>			
Caloric value (kcal/100g)	194	193.2	
Dry matter	97 ± 2	97 ± 2	96 ± 1.5
Fat	Negligible	Negligible	Negligible
Protein	Negligible	Negligible	Negligible
Ash	0.2	0.2	0.2
Sodium	Trace	Trace	Trace
Dietary fiber	88	88	
Reducing sugar	4.5	4.3	3.00
Vitamins, minerals	Negligible	Negligible	Negligible
<u>Color parameters</u>			
a*	-0.29 ± 0.014 ^a	-0.39 ± 0.014 ^b	-0.26 ± 0.00 ^a
b*	3.09 ± 0.00 ^a	2.77 ± 0.03 ^b	3.15 ± 0.02 ^a
L*	88.67 ± 0.03 ^c	92.24 ± 0.014 ^b	97.02 ± 0.03 ^a

L, value represents lightness from black (0) to white (100), a, value represents color ranging from red (+) to green (-); b*, value represents yellow (+) to blue (-); Means with different superscripts in a row is significantly different at $p \leq 0.05$ level.

present on the molecular chains are the major contributors towards the hydrophilic property. These hydroxyl groups can hydrate polymers due to interact with water molecules through hydrogen bonding.

2) Oil-holding capacity (OHC)

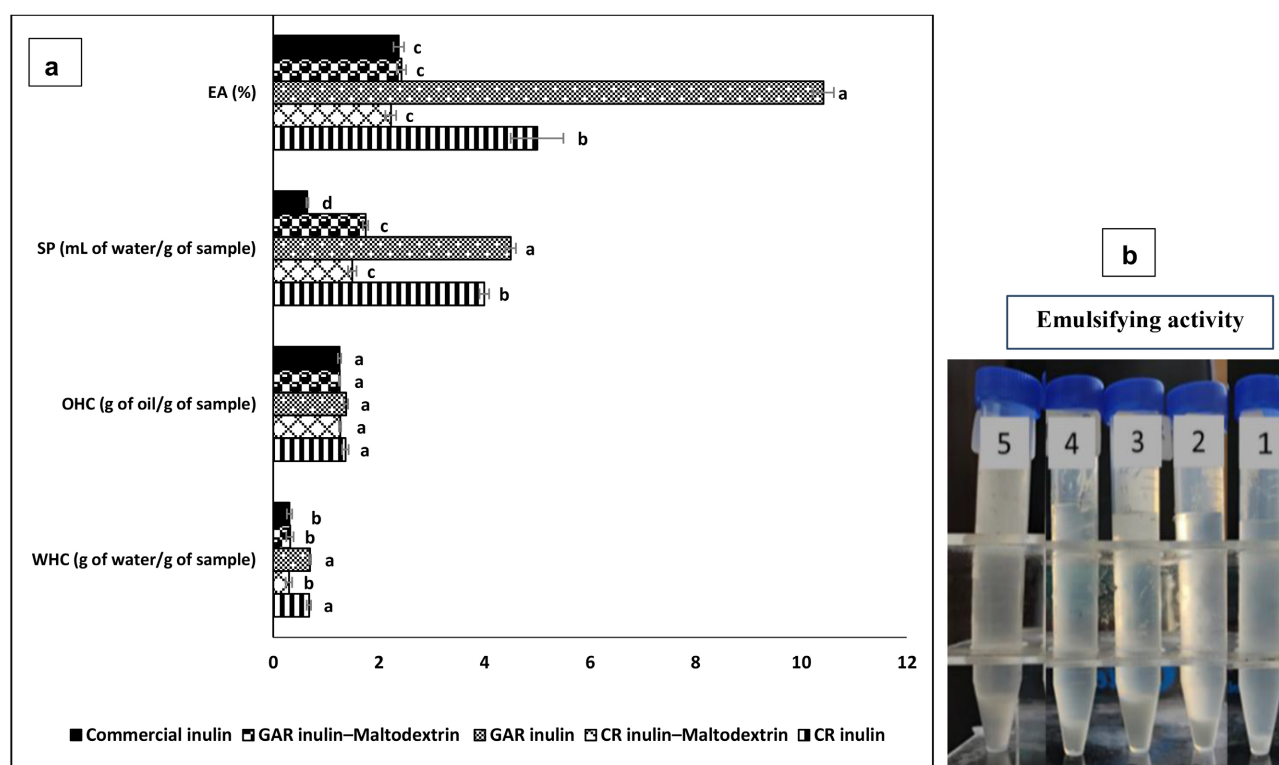


Figure 1. Functional properties of inulin. WHC: Water-Holding Capacity; OHC: Oil-Holding Capacity; SP: Swelling Power and EA: Emulsifying activity. 1: Commercial inulin; 2: GAR inulin-Maltodextrin; 3: GAR inulin; 4: CR inulin-Maltodextrin; 5: CR inulin).

The results indicated that no significant difference of OHC between all samples. The OHC of CRI and GARI had the highest values (1.37 and 1.38 g oil/g sample, respectively) as compared with that of CRIM and GARIM (1.27 and 1.26 g oil/g sample, respectively) and commercial inulin (1.24 g oil/g sample). The high OHC values of these inulin samples suggest that it can be used as a functional ingredient to improve the sensory properties of the formulated product, reduce syneresis, modify texture, modify viscosity, and reduce the calories of dairy products.

3) Swelling Power (SP)

Figure 1(a) shows that the swelling property of CRI and GARI had significantly ($P \leq 0.05$) the highest values (4.00 and 4.50 mL/g of sample, respectively) as compared with that of CRIM and GARIM (1.50 and 1.75 mL/g sample, respectively) and commercial inulin (0.65 mL/g sample). A Higher swelling property is mainly the function of increased surface area and low density. It was suggested that the differences in hydration properties were a function of the physical structure of maltodextrin and inulin. This could explain the differences in the hydration properties observed among CRI, GARI, CRIM, GARIM, and commercial inulin. Hydration properties determine the role of dietary fiber in regulating the colonic function and their physiological effects [25]. It is critical to control the amount of swelling of the hydrogels. High swelling of the gel is needed to allow degradation by bacteria in the colon.

4) *Emulsifying activity (EA)*

Emulsions are formed due to the presence of hydrophobic and hydrophilic groups of carbohydrates. **Figure 1(a)** and **Figure 1(b)** shows that the EA of the CRI and GARI was significantly ($P \leq 0.05$) higher (5.00% and 10.42%, respectively) as compared to the EA of CRIM and GARIM was (2.23% and 2.43%, respectively) and the EA of commercial inulin (2.38%). Probably, a relationship existed between the emulsion properties and solubility of the studied inulin and maltodextrin. This result suggests that in improving EA, it is proved that when inulin is applied in the gel shape, it can make more stable foam and emulsion, the surface microstructure of inulin contributes to its emulsion properties, WHC, and thickening properties [26]. However, the tested inulin demonstrated good EA values that met the requirements for it to become a beneficial food additive and showed its suitability for processing applications of functional dairy products.

3.2.3. Functional Groups

FTIR spectrometric analysis in the range of $4000 - 400 \text{ cm}^{-1}$ was used to identify the functional groups. **Figure 2** shows the FTIR spectra of standard, commercial, and CRI or GARI. Generally, the different inulin samples showed absorption peaks that were typical of standard inulin with bands for hydroxyl groups (O-H) at a wave number of 3366 cm^{-1} and 1440 cm^{-1} that were abundant in the molecular structure of inulin and presented by a very broad band were the most important functional groups detected by FTIR spectrometry [7]. An intense peak at 3366 cm^{-1} was observed from the stretching vibrations of OH due to inter and intra-molecular hydrogen bonds. The absorption sharp band at 2935 cm^{-1} was also observed. These bands were present due to the asymmetric C-H stretching vibration. The signals at 2115 cm^{-1} indicated the -C-C-stretch group. The peak at 1644 cm^{-1} that appeared was not specific for inulin and were attributed to the absorbance of water because of the hygroscopic properties of this homo-polysaccharide. The bands at 1224 cm^{-1} characterize of C-O-H bond. The bands at 1039 and 1132 cm^{-1} characterize of C-O and C-O-C stretching vibrations in the furanose ring, respectively. The band at the wave number 929 cm^{-1} was assigned to α -D-glucopyranosyl residue in the carbohydrate chain. Additionally, the bands at 874 and 817 cm^{-1} in the different inulin spectra confirmed the vibration of aromatics =C-H ring in the presence of 2-ketofuranose and 2-ketose. This indicated the presence of fructose with β -(2 \rightarrow 1) glycosidic bonds in different inulin samples. It was noted except in commercial inulin, that this phenomenon was absent in bands at 874 cm^{-1} (2-ketofuranose). The IR spectrum showed that the isolated substance from the different inulin-type fructan. Distinctive absorption bands at $542 - 595 \text{ cm}^{-1}$ and showed the presence of pyranose rings in a polymer chain.

3.2.4. Characteristics of SBY

1) *Physiochemical analysis of SBY*

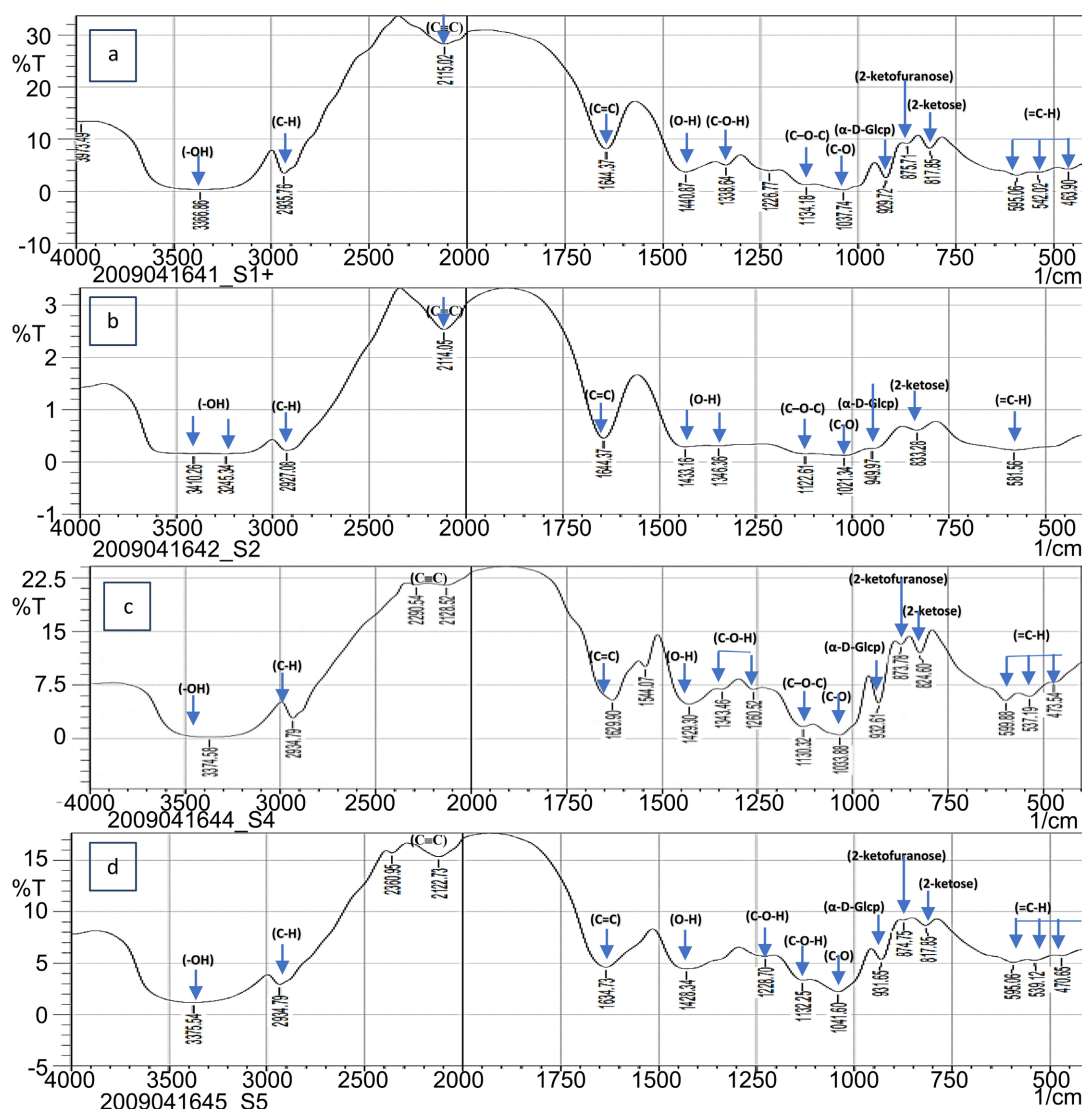


Figure 2. Functional groups of inulin (a) Standard inulin; (b) Commercial inulin; (c) CR inulin; (d) GAR inulin.

Table 2 shows that effect of fortification of inulin and maltodextrin as dietary fiber on the physicochemical properties of SBY during 21 days of cold storage. It can be observed that dietary fiber fortification significantly ($P \leq 0.05$) affected most of the physicochemical properties.

a) Total solid contents

The results obtained for the different treatments indicated significant differences ($P \leq 0.05$) in the total solid content. The addition of inulin slightly increased the total solid contents in the SBY samples at a range of 14.03% - 16.67%. The highest total solid was expressed by SBY that was fortified by CRI or GARI (T_2 and T_4) followed by SBY fortified by CRIM or GARIM (T_3 and T_5) treatment and the control. These results agree well with the findings of [15]. The results showed a significant increase ($P \leq 0.05$) of total solid during cold storage periods of all the treatments.

Table 2. Physiochemical analysis of fortified stirred bio-yogurt (SBY) during storage period.

Treatments (%)	Storage period (days)				
	Fresh	7	14	21	Means
Total solids					
C	14.03 ± 0.48	14.63 ± 0.31	14.97 ± 0.70	15.07 ± 0.76	14.68 ^C
T ₁	15.16 ± 0.38	15.49 ± 0.11	16.14 ± 0.34	16.37 ± 0.26	15.79 ^B
T ₂	15.65 ± 0.96	15.83 ± 0.17	16.39 ± 0.56	16.65 ± 0.30	16.13 ^{AB}
T ₃	15.39 ± 0.23	15.62 ± 0.17	16.24 ± 0.67	16.55 ± 0.15	15.95 ^{AB}
T ₄	15.73 ± 0.43	15.98 ± 0.05	16.46 ± 0.34	16.67 ± 0.14	16.21 ^A
T ₅	15.42 ± 0.89	15.78 ± 0.72	16.28 ± 0.46	16.60 ± 0.27	16.02 ^{AB}
Means	15.23 ^b	15.55 ^b	16.08 ^a	16.32 ^a	LSD = 0.405
pH value					
C	4.602 ± 0.11	4.531 ± 0.15	4.497 ± 0.30	4.432 ± 0.21	4.52 ^A
T ₁	4.594 ± 0.14	4.510 ± 0.23	4.484 ± 0.09	4.407 ± 0.00	4.50 ^A
T ₂	4.521 ± 0.07	4.385 ± 0.22	4.371 ± 0.31	4.352 ± 0.15	4.41 ^A
T ₃	4.562 ± 0.35	4.473 ± 0.21	4.449 ± 0.34	4.389 ± 0.00	4.47 ^A
T ₄	4.506 ± 0.06	4.378 ± 0.16	4.355 ± 0.05	4.320 ± 0.08	4.39 ^A
T ₅	4.540 ± 0.24	4.448 ± 0.18	4.410 ± 0.23	4.376 ± 0.16	4.44 ^A
Means	4.55 ^a	4.45 ^{ab}	4.43 ^{ab}	4.38 ^b	LSD = 0.156
Titratable acidity					
C	0.760 ± 0.08	0.913 ± 0.01	0.955 ± 0.04	1.030 ± 0.02	0.91 ^D
T ₁	0.815 ± 0.09	0.935 ± 0.07	1.027 ± 0.03	1.480 ± 0.05	1.06 ^A
T ₂	0.900 ± 0.02	1.083 ± 0.04	1.121 ± 0.02	1.228 ± 0.03	1.08 ^A
T ₃	0.865 ± 0.08	0.988 ± 0.05	1.091 ± 0.00	1.174 ± 0.02	1.30 ^C
T ₄	0.903 ± 0.00	1.095 ± 0.05	1.145 ± 0.03	1.243 ± 0.00	1.10 ^A
T ₅	0.885 ± 0.01	1.029 ± 0.00	1.115 ± 0.03	1.187 ± 0.01	1.05 ^C
Means	0.85 ^d	1.007 ^c	1.08 ^b	1.22 ^a	LSD = 0.034
Synaeresis (%)					
C	33.57 ± 0.23	34.44 ± 0.43	37.44 ± 0.31	39.47 ± 0.41	36.23 ^A
T ₁	29.16 ± 0.25	31.96 ± 0.19	33.83 ± 0.37	35.32 ± 0.85	32.57 ^B
T ₂	23.96 ± 0.68	27.98 ± 0.68	30.62 ± 0.55	30.59 ± 0.88	28.29 ^E
T ₃	27.82 ± 0.54	31.74 ± 0.68	32.93 ± 0.87	34.24 ± 0.58	31.68 ^C
T ₄	23.45 ± 0.77	25.40 ± 0.68	28.68 ± 0.61	29.35 ± 0.57	26.72 ^F
T ₅	26.93 ± 0.74	30.26 ± 0.87	32.43 ± 0.72	33.16 ± 0.58	30.70 ^D
Means	27.48 ^d	30.30 ^c	32.66 ^b	33.69 ^a	LSD = 0.523
Color parameters	L*	a*	b*		
C	89.08 ± 0.90 ^A	−1.47 ± 0.05 ^B	8.48 ± 0.02 ^B		
T ₁	88.72 ± 0.02 ^B	−1.51 ± 0.02 ^B	8.73 ± 0.08 ^A		

Continued

T ₂	85.48 ± 0.04 ^F	-1.25 ± 0.07 ^A	6.20 ± 0.01 ^E
T ₃	87.39 ± 0.03 ^D	-1.50 ± 0.04 ^B	7.54 ± 0.07 ^C
T ₄	86.74 ± 0.05 ^E	-1.43 ± 0.06 ^B	6.94 ± 0.05 ^D
T ₅	87.95 ± 0.08 ^C	-1.72 ± 0.03 ^C	7.64 ± 0.04 ^C

C: Control SBY without addition; T₁: SBY fortification with Commercial inulin; T₂: SBY fortification with CR inulin; T₃: SBY fortification with CR inulin-maltodextrin; T₄: SBY fortification with GAR inulin and T₅: SBY fortification with GAR inulin-maltodextrin. *L, value represents lightness from black (0) to white (100), a*, value represents color ranging from red (+) to green (-); b*, value represents yellow (+) to blue (-). Means with different superscripts in a row or column are significantly different at $P \leq 0.05$ level; Data represented as means of triplicates \pm SD; LSD = Least significant differences at 0.05% level.

b) pH and titratable acidity

The addition of inulin in milk until the preparation of SBY increases the acidification speed and decreases pH and fermentation time. This result agrees with the previously reported results by [27]. The pH values and titratable acidity of SBY from different treatments had slightly significant effects by different sources of inulin during 21 days of storage.

The pH values and titratable acidity of the SBY samples were not affected by different sources of inulin. The inulin fortification led to a decreased pH and increased titratable acidity of SBY as compared to control ($P \leq 0.05$). SBY (T₂ and T₄) containing CRI or GARI recorded significantly lower pH and higher ($P \leq 0.05$) titratable acidity than SBY (T₃ and T₅) containing CRIM or GARIM. Similar observations were presented by [8]. Inulin contains soluble fiber that might have been easily digested by the bacteria resulting in the formation of by products such as organic acids [28] including lactic acid that leading to increased titratable acidity and decreased pH.

c) Synaeresis

The results indicated that the addition of recorded inulin significantly ($P \leq 0.05$) reduced the synaeresis in all the treatments as compared to the control. This may be attributed to the interaction between the inulin and the milk portion. These results agree well with the findings of [29] where they added inulin to yogurt due to bond formation with casein and improvement in the protein network stability cause a reduced synaeresis, the decreased syneresis is attributed to the WHC of inulin dietary fiber that absorbed the whey released by the gel structure. SBY (T₄ and T₂) had obtained lowest levels from synaeresis as compared to that from SBY (T₅ and T₃) and commercial inulin (T₁) due to maltodextrin easily dissolves in water with low hygroscopicity and high-water solubility and forms sticky solutions, but the addition of inulin improves WHC for treatment. Additionally, CRIM or GARIM yielded the same results of commercial inulin. Nevertheless, there is a significant increase ($P \leq 0.05$) of syneresis during the storage of all treatments. [29] reported that the addition of inulin in

the fermented product resulted in the lower syneresis index and a greater firmness and cohesiveness.

d) Color analysis

Table 2 shows the changes in the color parameters (L^* , a^* , and b^*) in fresh SBY treatments. The L^* value for the control (C) and commercial inulin SBY were significantly higher than the L^* values for all the SBY treatment. The a^* and b^* values for all the treatments were significantly different from each other.

e) Apparent viscosity

Figure 3(a) and **Figure 3(b)** shows the effects of shear rate on the apparent viscosity of fresh SBY and after 21 days of storage. The viscosity of all the samples was reduced after increasing the shear rate. All treatments fortified with inulin or inulin-maltodextrin exhibited the highest values of viscosity as compared to the control. [30] proved that reconstituted milk that was fortified with 2% inulin showed a higher viscosity score as compared to the non-fat milk sample. There were differences between the different treatments on the SBY viscosity. The results indicated that SBY (T_5 and T_3) and the commercial inulin (T_1) had the highest values of viscosity as compared to SBY (T_4 and T_2) due to the high viscosity of maltodextrins which is an important property that is attributed to the high levels of high-molecular weight saccharides in many applications. An increase in viscosity was observed due to increment in the TS and the high water-retaining capacity of inulin. In some studies, the increase in viscosity due to the addition of dietary fiber has been attributed to the interactions between inulin and dairy proteins [31]. Inulin has some functional properties such as its WHC, texture modification, emulsifying activity, and/or gel formation, which affect on the yogurt viscosity. Inulin gel formation is different from that obtained with hydrocolloids. Inulin forms particle gels, whereas the increase of viscosity through most hydrocolloids is obtained by the bonds between the chains. Nevertheless, there is an increase of viscosity during storage of all the treatments. [32] proved that inulin has an affirmative effect on syneresis (decreasing) and apparent viscosity (increasing) in fat-free yogurt. The viscosity of all fortified SBY samples showed a slight increase in viscosity after 21 days of storage. This may be attributed to the fact that the SBY viscosity was affected by the number, strength, structure, and spatial distribution of casein micelle bonds enhanced by storage.

2) Microstructural characterization

Figures 4(a)-(f) shows the microstructural characterization of different SBY samples. The micrograph (a) shows, that the control SBY appeared as a more open and irregular protein network as consequence of the lower number of fat globules acting as the linking protein agents potentially and negatively affected its microstructure, further culminating in a weaker network structure [33] and less attractive textural properties than other treatments. These aspects can be related with control SBY textural properties, such as increased spontaneous syneresis and low viscosity values (**Table 2** and **Figure 4**).

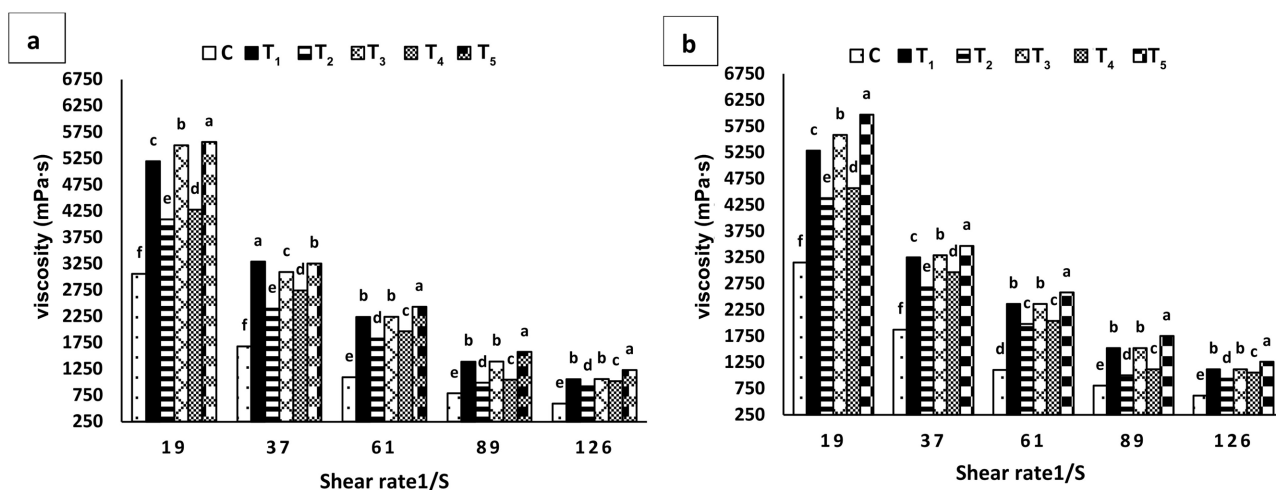


Figure 3. Apparent viscosity of fortified stirred bio-yogurt (SBY) at different shearing rates. (a) at fresh; (b) after; 21 days from storage period. C: Control SBY; T₁: SBY fortification with Commercial inulin; T₂: SBY fortification with CRI; T₃: SBY fortification with CRIM; T₄: SBY fortification with GARI and T₅: SBY fortification with GARIM.

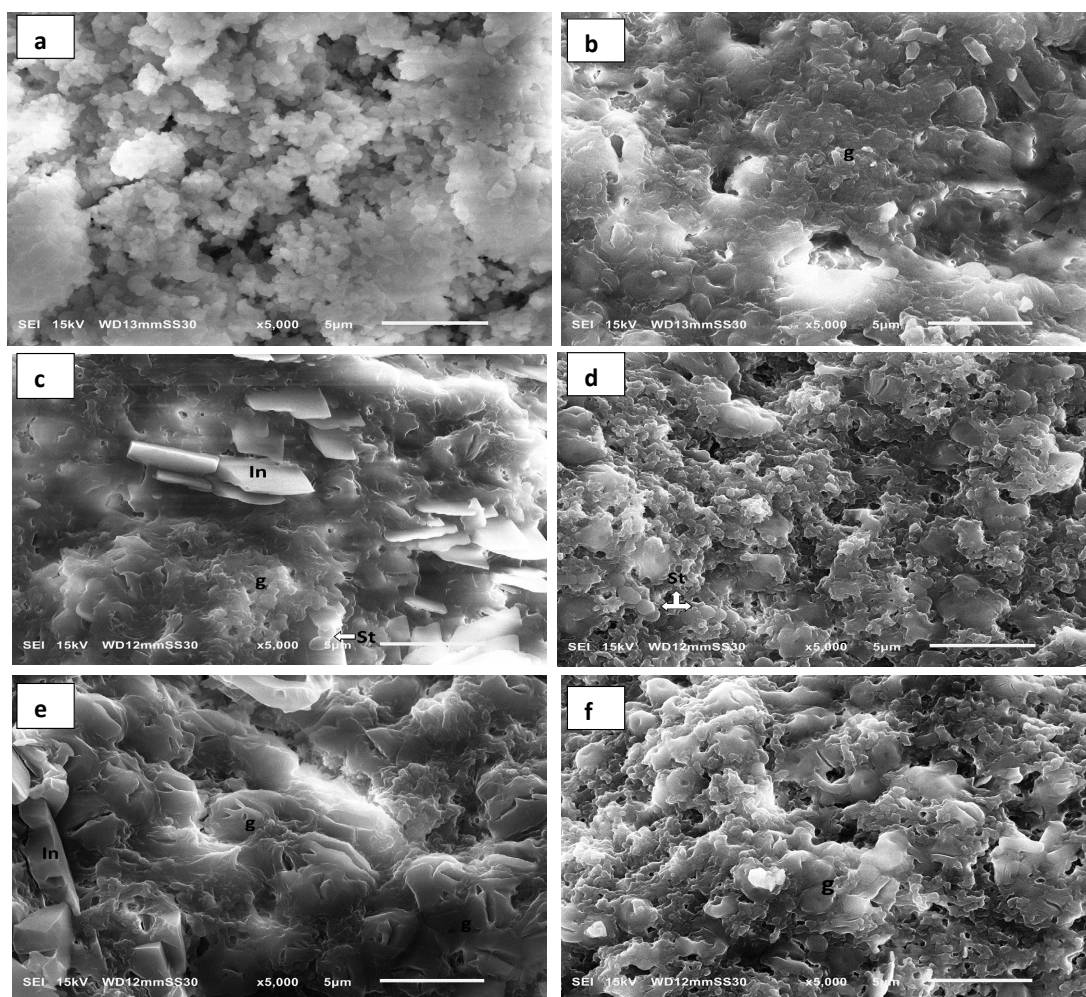


Figure 4. Microstructure of fortified stirred bio-yogurt (SBY) produced without any addition (control) (a), and with commercial inulin (b), and with CR- and GAR-extracted inulin (c and e), with maltodextrin-CR- and GAR-extracted inulin (d and f), (g): inulin gelled structures, In: Inulin, and St: *Streptococcus thermophilus*.

All the treatments contain inulin exhibited a denser gel structure (g) with more interconnected protein clusters, containing smaller and more homogeneous void spaces. The micrograph (c, e) and (b, d, f) shows that the protein network affects the microstructures of SBY ($T_{2,4}$) and SBY ($T_{1,3,5}$). It appears that inulin has to be the part of the structural network aggregates via a hydrogen bond. Furthermore, improving milk proteins network stability leads to reduced syneresis that contributed in the yogurt network structure [34], which is formed by the elongated gelled structures that intermingled into the protein network by acting as a water-structuring agent hence, it acts as a thickener. It is important to mention that [35] revealed that inulin formed a secondary network of casein micelles in the low-fat SFY and helps to more consistent gel formation.

Additionally, there was a noticeable similarity between the SBY microstructure fortified with CRI or GARI in micrograph (c and e). This similarity was also observed between commercial inulin and CRIM or GARIM in micrograph (b, d, and f), thereby demonstrating that the yogurt gel organization was not affected by the different sources of inulin utilized.

3) Microbiological analysis

Figure 5 shows the changes in the viable counts of survival probiotic cultures *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* for the different SBY samples during storage.

In the fresh samples, the numbers of all probiotic cultures in the presence of inulin in SBY are higher than the control sample due to the addition of inulin had a positive effect on the survival of probiotic cultures. These increased probiotic counts of yogurt are attributed to inulin acting as a prebiotic substance. These results agree with [36] who noted that the functionality of inulin as a food additive is mainly referred to its prebiotic properties such as the ability to stimulate probiotic microorganisms without adversely affecting the flavor.

The count of probiotic cultures showed a slight decrease during the storage for all the treatments and control. Notably, the viable probiotic culture of all treatments and control exceeded above 10^6 CFU/g during the storage up to 21 days, which render these yogurts to be bio functional products containing the recommended count of viable probiotics (10^6 CFU/g) and fortified with inulin prebiotic. Inulin exerts a protective effect on the SBY culture and probiotics by increasing their survival and activity during storage [37].

Moreover, coliforms, yeast, and mold were not detected in any treatments either when they were fresh or throughout the storage period (data not shown). However, yeast and mold were detected after 21 days of storage in the control samples.

4) Sensory evaluation

Figure 6 shows the effects of different sources of inulin or inulin-maltodextrin as dietary fiber on the sensory properties of SBY as compared to plain control during storage. Overall, the acceptability scores of SBY samples fortified with CRI or GARI showed a great score followed by CRIM or GARIM and the commercial

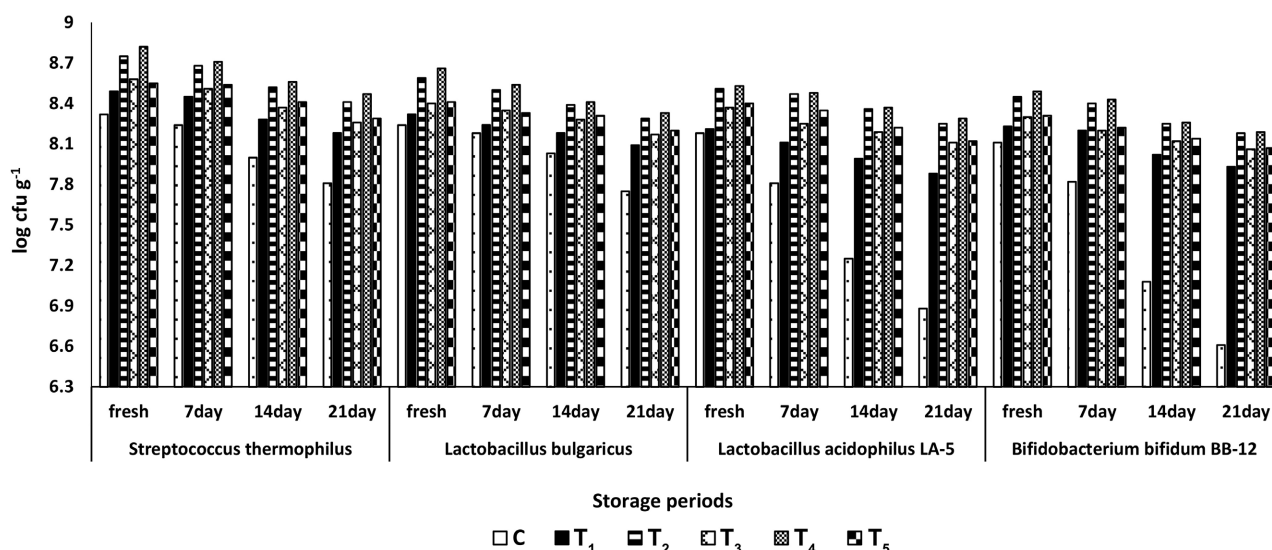


Figure 5. Microbiological analysis of fortified stirred bio-yogurt (SBY) ($\log \text{cfu g}^{-1}$) during storage. C: Control SBY; T₁: SBY fortification with Commercial inulin; T₂: SBY fortification with CRI; T₃: SBY fortification with CRIM; T₄: SBY fortification with GARI; T₅: SBY fortification with GARIM.

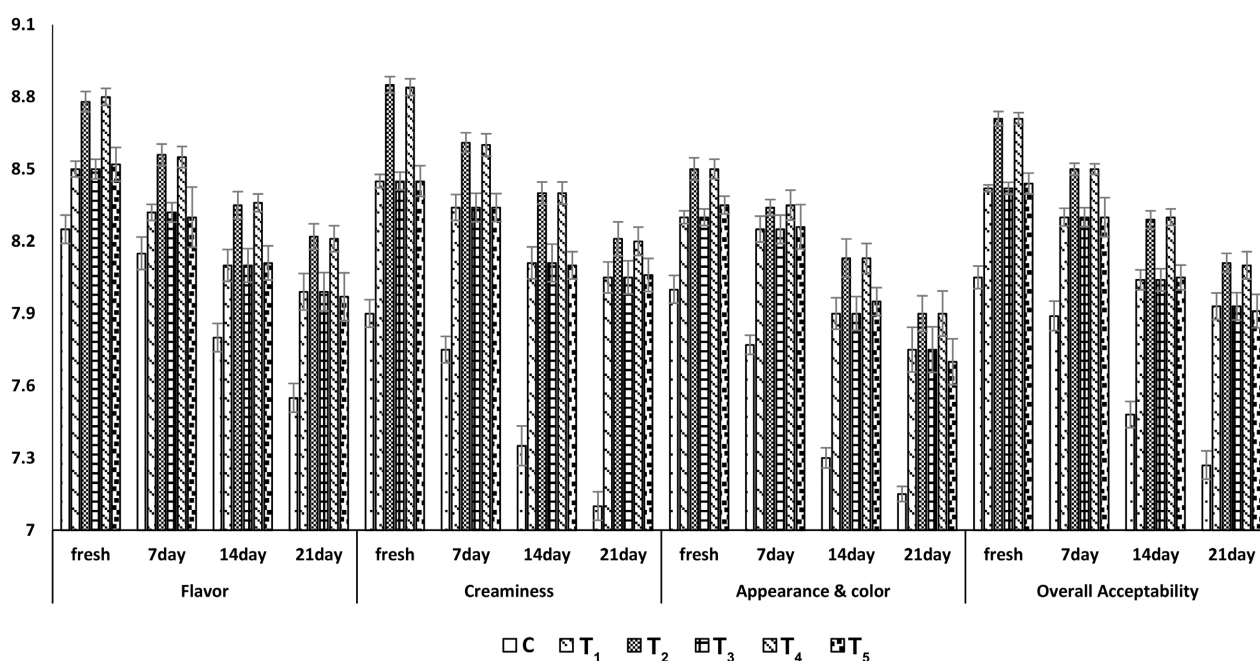


Figure 6. Sensory evaluation of fortified SBY during storage. C: Control SBY; T₁: SBY fortification with Commercial inulin; T₂: SBY fortification with CRI; T₃: SBY fortification with CRIM; T₄: SBY fortification with GARI; T₅: SBY fortification with GARIM. Data represented as means of duplicates \pm SD.

inulin as compared to the plain control SBY. It was clear that the addition of inulin or inulin-maltodextrin improved the sensory evaluation of fresh samples. This is related to a reduction in the properties of creaminess score as compared to other treatments due to the decreased fat content of control SBY leads to a loss of texture and sensorial property [3]. The sensory properties results indicated that the addition of inulin in SBY increases creaminess as compared to the

control, due to less solubility and high ability of bond forming. It could also be used as the replacement of milk fat in SBY without modifying color, off-flavor and improving the appearance attributes [34]. The sensory parameters showed less changes during 21 days of cold storage in the samples fortified with inulin or inulin-maltodextrin. These results indicate that the inulin effectively reduced the negative changes in flavor, thus improving the sensory properties.

4. Conclusion

Presently, there is an increased consumers' attention toward the diet and daily calories intake. Extracted, purified, and produced inulin from CR and GAR showed a high yield and purity. These products can offer various health benefits and wide applications as supplement of prebiotic dietary fiber in the dairy industry. They can also be used in manufacturing different functional healthy dairy products and food.

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Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Consent to Participate

All authors consent to participate (were responsible for the conceptualization, experimental design, methodology, performed formal analyses, wrote the original draft of the manuscript, revised, edited, and confirmed the manuscript).

Consent for Publication

All authors consent for publication (data or image curation and analysis).

Availability of Data and Material

All data and materials were availability.

Authors' Contributions

All authors contributed equally to this research work. W.M.E., G.H.B. and R.H.A. were responsible for the conceptualization, experimental design, methodology, performed formal analyses, data curation and analysis. All authors wrote the original draft of the manuscript, revised, edited, and confirmed the manuscript.

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