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Enhancement of the quality attributes and health benefits synbiotic yoghurt from cow's milk

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ABSTRACT

The present work delighted on extraction of galactomannan polysaccharide from guar gum beans and microbial galactomannan source. Effect of replacing non-fat dry milk that used to fortify cow's milk in yoghurt industry with the two extracted galactomannans and commercial galactomannan as food additives was studied. Control yoghurt treatment was made from 3.0% fat cow's milk that was fortified with 1.5% non-fat dry milk. Another 6 yoghurt treatmentwas fortified with 0.15, 0.25% of commercial, guar and microbial galactomannan respectively. All treatments were cultured with the probiotic starter (1.0% *Streptococcus thermophilus* + 1.0% *Lactobacillus delbrueckii* subsp. *Bulgaricus* + 1.0% *Bifidobacteriumbifidum*). The obtained results indicated that yoghurt supplementation with the three types of galactomannans increased the acidity, curd tension, total solids content, decreased pH values and syneresis of yoghurt treatments. Control yoghurt and commercial galactomannan yoghurt were not significantly different from the corresponding batches those made with either guar galactomannan and microbial galactomannan in fat, protein and ash content. Yoghurt treatments which supplemented with the three types of galactomannans have higher bifidobacteria counts and organoleptic scores than the control treatment yoghurt.

1. Introduction

Guar plant is a leguminous plant that grows up to 3–6 feet, produces many 5–12.5 cm long bean-like pods in clusters with 6–9 small seeds per pod (Fig. 1). Extremely drought tolerant annual crop that can be cultivated with very limited supply of resources.

to be fig. 1 Guar plant and seeds here.

Galactomannan polysaccharide extracted from guar beans that has thickened and stabilizing properties useful in food, paper, textile, feed, and industrial applications. Galactomannan used as a stabilizer in frozen (ice cream) and baked foods. Thickener for salad dressing due to high viscosity, acid stability cold water dispersibility. It has been used to reduce cholesterol and blood glucose level. In the pharmaceutical industry, used as a binder or as disintegrator in tablets; bulk-forming laxatives. Galactomannan chemically is an exopolysaccharide composed of galactose and mannose units. Galactomannan is classified as dietary fiber that remains undigested in the human digestive tract after consumption. Gums can have favorable effects on human physiology, such as lowering glycemic

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Fig. 1. Guar plant and seeds here.

response and cholesterol levels in the blood [1]. Guar galactomannan participate in physiological processes such as diabetes control through blood glucose levels, heart disease control through blood cholesterol reduction, and a healthy digestive system through nutrient absorption and bowl movement regulation. When galactomannan is disseminated in a liquid such as water, it creates a viscous solution. It is employed as a thickening and stabilizing in a range of meals due to its high viscosity in aqueous solution [2].

As people become more conscious of health issues, they consume more functional dairy products that contain nutritional supplements. Foods fortified with dietary fiber generally result in low-calorie, low-cholesterol, and low-fat foods. Incorporating dietary fiber food products may also increase their functional characteristics [3]. Dietary fiber is made up of plant carbohydrate polymers such as oligosaccharides and polysaccharides that are indigestible in the human small intestine, but ferment completely or partially in the large intestine. Dietary fiber is divided into two types: soluble and insoluble. Pectic compounds, gums, mucilage, and inulin are soluble fibers, whereas cellulose, hemicellulose, and lignin are insoluble fibers. When consumed in the diet, dietary fiber can perform one or more activities, such as increasing fecal bulk, stimulating colonic fermentation, lowering blood glucose, and lowering cholesterol levels [4].

Yogurt is a fermented dairy product with high nutritional and health benefits. Food scientists have attempted to increase the nutritional benefits of yoghurt by adding protein and fiber, as well as assesses the functional and sensory aspects of yoghurt [5].

Food and Agriculture Organization (FAO) and World Health Organization (WHO) define probiotics as live micro-organisms (bacteria or yeasts), when ingested or locally applied in sufficient numbers discuss one or more specified demonstrated health benefits [6]. Bifidobacteria as probiotic bacteria show antagonistic effects towards some pathogens, reduce the risk of diarrhea, normalize the bowel movement, enhance immune functions, reduce cholesterol level, reduce the risk of eczema, synthesize several vitamins, protect from cancer and relieve of lactose intolerance symptoms [7]. There has been a growing interest in employing bifidobacteria as adjuncts in the dairy sector as a result of these possible roles. Probiotic organisms, on the other hand, are unstable in such preparations. Its viability may have been lost due to acidity, freezing injury, or oxygen toxicity [7–9]. The effectiveness of probiotic bacteria introduced at a higher dose is dependent on the dose level. Their viability must be maintained throughout the shelf life of the product, as well as their capacity to live in the gut environment. They must establish themselves in significant quantities in the gastrointestinal tract to exercise their health effects [10]. A bifidobacteria standard growth that requires at least of $10^6 - 10^7$ colony forming units per gram (cfu/g). Several food organizations throughout the world have made fermented milk products available [10–12]. It is well known that making yoghurt from cow's milk has a weak body and texture. Therefore, it has been recommended to fortify cow's milk with non-fat dry milk and some stabilizers [13].

In view of the aforementioned the objectives of this study were to investigate the possibility of reducing the amount of non-fat dry milk that used to fortify cow's milk by adding galactomannan from different sources as a texture modifier during manufacture of probiotic yoghurt and to monitor the changes during storage period.

2. Material and methods

2.1. Chemical analysis of guar gum beans

The seeds of *Cyamopsistetragonoloba* (guar gum beans) were purchased from local market and milled using high speed blender (IKA-Laboratechnic, Germany) to be used as sources of galactomannan. Chemical properties of moisture, ash, crude protein and lipids were determined according to (AOAC, 2000) [14]. Total carbohydrates were determined after complete acid hydrolysis [15].The resulted acid hydrolysates were examined by PC using n-BuOH-MeCO-H_2O (4:5:1) [16] and aniline phthalate [17] as spraying reagents. Quantitative determination of the separated sugars was carried out according to [18]. Total nitrogen of the investigated samples was determined by adopting the usual micro-Kjeldahl's method [14]. The crude protein was calculated by multiplying the total nitrogen by 6.25 [19].

2.2. Extraction and characterization of galactomannan

Extraction process was carried out by Jindal and Mukherjee [20] with some modification by using hot water at pH7. Briefly,

defatted powdered plant material (5 g) was extracted with 200 ml of extracting hot water at 85 °C for 3 h. After filtration, the extract was neutralized and dialyzed against distilled water for 48 h., dried and weighed. The chemical characterization of the extracts was achieved by determining their total carbohydrates and the monosaccharide constituents of the extracts hydrolyzed. The methods used for this analysis were previously mentioned [18,21]. Soluble protein was determined by the Lowry method [22]. Yeast galactomannan prepared according to Edwards [23] with some modification extraction with 6 N NaOH (at 100 °C for 2 h crumbled yeast)followed by neutralization, removal of debris, and precipitation of polysaccharide with methanol is the most commonly used method. The galactomannans precipitate as the copper complex very slowly, requiring as long as two or three months at 3–4 °C.

2.3. FT-IR spectroscopy

FT-IR (Bruker Vectra 22) Spectrometer equipped with a Dura Sample IR II™ detector used for characterization and identification of commercial, guar and microbial extracted glactomannan powder with a spectral resolution of 4 cm^{-1} with 400–4000 cm^{-1}.

2.4. Scanning electron microscopy (SEM)

The surface morphology of three glactomannan samples was examined using scanning electron microscopy (JEOL 5410) microscope with an accelerating voltage conducted at 20 kV. Galactomannan samples were gold coated using a Hitachi coating unit IB-2 coater under a high vacuum, 0.1 Torr, high voltage, 1.2 kV and 50 mA.

2.5. Energy dispersive X-ray spectroscopy

EDAX (or EDS) is an x-ray spectroscopic method for determining elemental compositions (qualitative and quantitative analysis). It can be used with/during imaging with SEM. When done with an SEM instrument, the signal can be acquired from a spot, an area, a line profile or a 2D map.

2.6. Prebiotic activity

Prebiotic activities of commercial and two extracted galactomannanwere evaluated. The three probiotics *L. Casei, L. reuteri* and *L. Helveticus* were grown in the MRS medium, while *E. coli* was grown in nutrient broth medium, at 37 °C for 24 h. Aliquots of 0.1 ml of each of the resulted bacterial cultures were used as inoculum for 10 ml studied medium supplemented with 150 mg studied samples as carbon source. After incubation at 37 °C for 24 h, the resulted bacterial growth was measured at 625 nm against a blank of an inoculated medium [24]. The prebiotic activity was calculated as "Prebiotic Index" (I):

Prebiotic index = (Optical density of probiotic culture at 600 nm/Optical density of *E. coli* culture at 600 nm) x 10.

2.7. Bacterial strains and propagation

Active *Streptococcus thermophiles* EMCC 1043, *Lactobacillus delbrueckii* subsp. *bulgaricus* EMCC 1102, *Bifidobacterium bifidum* DSM 20082 were obtained from Cairo Mercin, Ain Shams University, Egypt. Yoghurt starter strains *Streptococcus thermophilus* EMCC 1043 and *Lactobacillus delbrueckii* subsp. *Bulgaricus* EMCC1102 were activated individually by three successive transfers in MRS broth, then were activated individually by three successive transfers in sterile 10% reconstituted non-fat dry milk. Bifidobacteria strain *(Bifidobacterium bifidum*) was activated by three successive transfers in modified MRS broth medium [25], and incubated at 37 °C under anaerobic conditions using gas pack (OXOID Ltd, Basingstoke, Hampshire, England).

2.8. Manufacture of yoghurt

Fresh cow's milk was obtained from local markets, Menoufiya governorate, Shibin El-Kom, Egypt. Milk was standardized to 3.0% fat. Seven yoghurt treatments were made. The preliminary experiment showed that the best yoghurt quality was made by supplementing cow's milk with 1.5% non-fat dry milk. Control treatment (C) was made from 3.0% fat cow's milk supplemented with 1.5% non-fat dry milk (Fresno, California, USA). Two yoghurt treatments (T_1 and T_2) were fortified with 0.15 and 0.25% of commercial galactomannan, respectively. Another two yoghurt treatment was made from the same cow's milk, but by adding 0.15 and 0.25% of guar galactomannan (T_3 and T_4, respectively. The other two yoghurt treatments were made as described previously except that they fortified with a rate of 0.15 and 0.25% of microbial galactomannan (T_5 and T_6), respectively. Non-fat dry milk, commercial galactomannan, guar galactomannan and microbial galactomannan were added to milk and stirred thoroughly during heat treatment, then filtered through cheesecloth. All milk treatments were heated to 85 °C for 20 min, then cooled to 42 °C and inoculated with 1.0% *Streptococcus thermophilus* + 1.0% *Lactobacillus delbrueckii* subsp. *Bulgaricus* + *1.0% Bifidobacteriumbifidum*. The inoculated batches were packed in plastic cups and incubated at 42° until complete coagulation. All yoghurt treatments were stored in the refrigerator (6 °C ± 1) for 12 days and were sampled when fresh and at 3, 6, 9 and 12 days for chemical, rheological analysis and sensory evaluation. The whole experiment was triplicate.

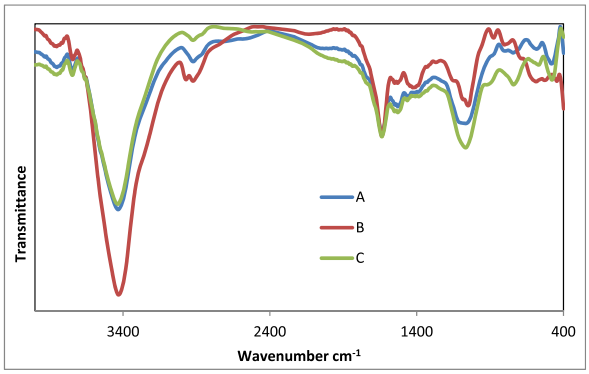


Fig. 2. FT-IR spectroscopy of guar (A), microbial (B) and commercial galactomannan (C).

2.9. Chemical analysis

pH values, titratable acidity and fat content were determined according to Ling [26], while total solids, ash and total protein were determined according to the methods described by AOAC [14].

2.10. Rheological properties

Wheysyneresis was determined according to the method of Dannenberg and Kessler [27] with slight modification. A hundred grams of yoghurt plastic cups were cut into four sections and transferred into a funnel fitted with 120 mesh metal screen. The amount of whey drained into a graduate cylinder was measured after 120 min, at room temperature (20 $\pm$ 1 °C) for all yoghurt samples stored for 1, 3, 6, 9 and 12 days. The curd tension of yoghurt was assessed using nondestructive Effagi firmness measurements (Effagi, Albonsine, Italy). The penetration depth was 50 mm using a stainless steel plunger flat ended with a diameter of 5 mm. Five readings were taken for each yoghurt treatment.

2.11. Sensory evaluation

Yoghurt samples were judged by panelists from the staff members of Dairy Science and Technology Department, Faculty of Agriculture, Menoufiya University. Results were recorded on a score sheet described by Kebary and Hussein, [28]. The panelists were subjected to sensory evaluation using hedonic scale for flavor, body & texture, appearance, acidity and total scores. The experiments were approved by the Scientific Research Ethics Committee (SREC) – Faculty of Agriculture – Menoufia Universty (Approval number: 01-SREC-MUAGR-07-2023).

2.12. Bacteriological analysis

Samples from each yoghurt was taken when fresh and after 1, 3, 6, 9 and 12 days of refrigerated storage for counting bifidobacteria. The modified MRS agar was used for enumerating bifidobacteria [25]. For each 100 ml of modified MRS 5.0 ml of the following solutions were added before pouring plates Neomycine sulphate (0.8% w/v), Paromycine sulphate (0.2% w/v), Nalidixic acid (0.3% w/v) and Lithium chloride (6.0% w/v)) [29]. Plates were incubated under anaerobic condition using gas Pa ck (OXOID Ltd, Basingstoke, Hampshire, England) at 37 °C for 72 h.

2.13. Statistical analysis

Data were analyzed using completely randomized block design and 2 $\times$ 3 factorial design. Newman Keul's test was used to make the multiple comparisons [30] using Costat program. Significant differences were determined at $p \leq 0.05$.

3. Results

3.1. Chemical analysis of guar seeds

The results showed the percentage composition of guar seeds. The ash content 5.4%, polymeric carbohydrate 54.2%, protein 38.3%, and lipid 2.6% agreed with Yousif [31]. The results of these chromatographic investigations revealed the presence of galactose, glucose, mannose and arabinose as structural units of the polymeric seed carbohydrates. However, traces of other 2 sugar components, i.e., xylose and glucuronic acid, were also detected as constituents of the seed hydrolyzates. Galactose 39.4%, glucose 31.4%, mannose 17.4%, arabinose 11.7%, xylose and uronic acid traces.

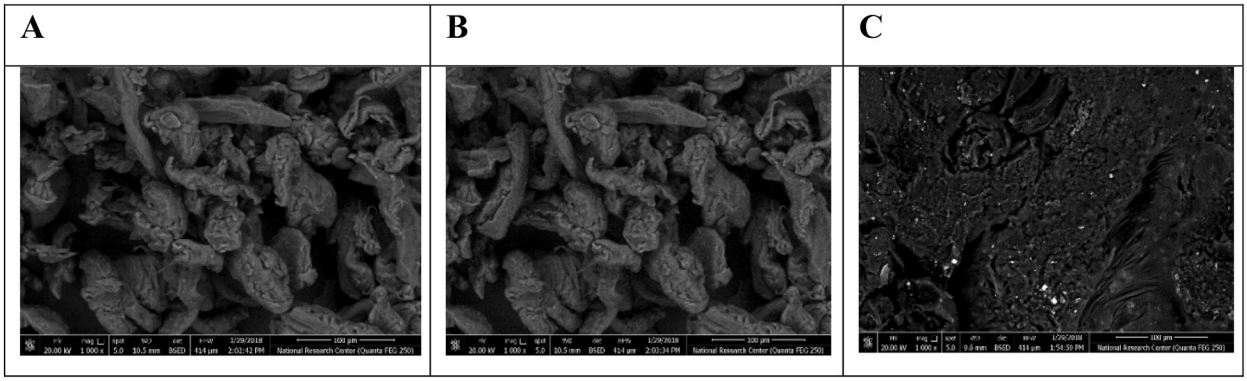


Fig. 3. SEM image of commercial (A), guar (B) and microbial (C) galactomannan.

3.2. Galactomannan phisyochemical analysis

Guar and microbial galactomannan yield was 7.4% and 3.6%. Guar, microbial and commercial galactomannan ash 0.5%, 0.41% and 0.21%, total carbohydrate (T.C) 95.5%, 93.4% and 94.5% and protein content 4.8%, 3.9% and 4.1%, respectively. Chromatographic analysis of the acid hydrolysates of galactomannan samples revealed the presence of glucose, arabinose and glucuronic acid in addition to galactose and mannose, the building units of galactomannan chains. Furthermore, quantitative determination of the aforementioned monosaccharide components indicated that the majors are galactose and mannose while the other sugars are the minors. Guar, microbial and commercial galactomannan indicated monosaccharides as mannose 55.6%, 57.1%, 48.9%; galactose 36.9%, 34.5%, 38.6%; glucose 5.4%, 5.2%, 8.7%; arabinose 1.3%, 1.5%, 1.8% and glucuronic acid (traces), respectively. Mannose/Galactose ratio for guar, microbial and commercial galactomannan was 1.51, 1.65 and 1.26, respectively. Physicochemical characteristics of the polysaccharide extracted from legume *Delonixregia* seed were originated to consist of mannose and galactose with an Mannose/Galactose ratio 5:1 [32].

3.3. FT-IR spectroscopy

FT-IR glactomannans spectrum represented a variety of bands ranging from 3450 to 400 cm^{-1} (Fig. 2). The commercial, guar and microbial extracted glactomannan have the same spectrum with minor changes. All three glactomannan types has a broad absorption at 3400–3500 cm^{-1} corresponding to the stretching vibration of the –OH group of carbohydrates in presence of moisture. However, guar extracted glactomannan has sharper absorption band than commercial and microbial type. The three glactomannan types also exhibited an absorption at 2920 cm^{-1} conforming to stretching vibration of C–H group. Characteristic band at 1620 cm^{-1} caused by stretching C═C and amide groups, which confirmed the protein presence in galactomannan moieties [33]. Whereas, peak at 1100 cm^{-1} indicating C–O and C–*O*–C bonds. Peaks at 850-800 cm^{-1} described the existence of C–H oscillations ofβ and α conformers, qualified to β-D-mannopyranose and α-D-galactopyranose units, respectively [34]. The above explained peaks have been attributed to carbohydrate biopolymer in literature [35].

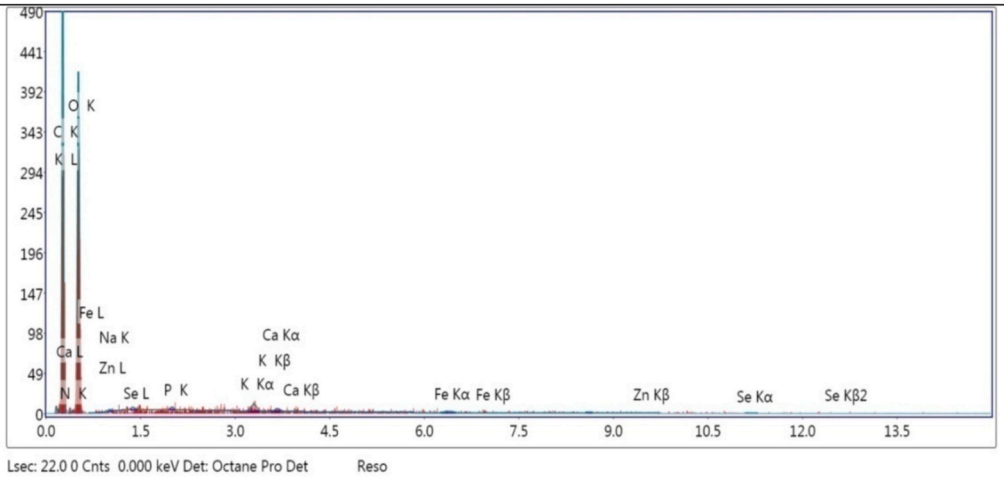
3.4. SEM analysis

SEM analysis gives information about the external morphology texture through two-dimensional image. The images presented surface morphologies of commercial, gear and microbial extracted glactomannan were taken at the same magnification (20.00 K X) and scale of 100 μm. Commercial and guar extracted glactomannan images present large aggregates with a rough surface (Fig. 3A and 3B).The aggregates have no certain shape with different sizes that are grouped together. The microbial glactomannan image appeared to be formed a smooth surface covered by several holes (Fig. 3C).

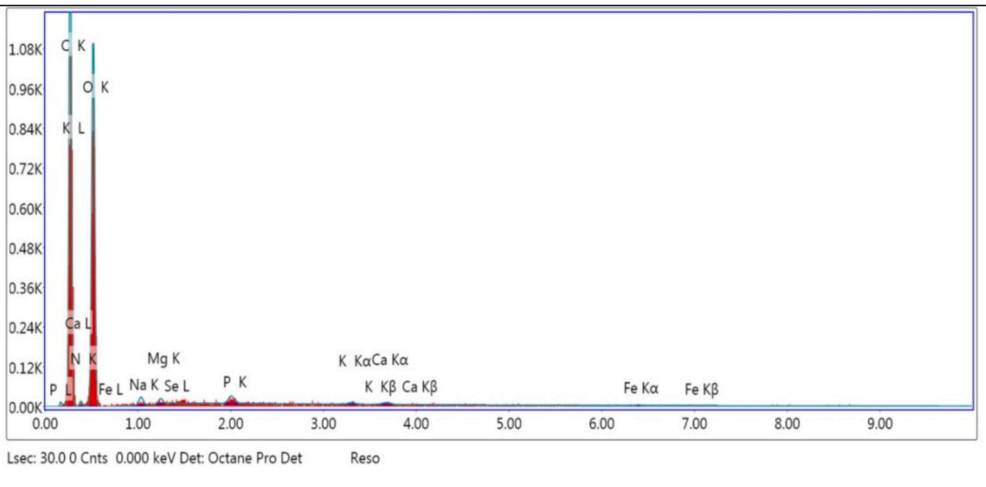
3.5. EDAX analysis

EDAX is a chemical Microanalysis method used in conjunction with SEM analysis to determine qualitative and quantitative elements on the surface of the commercial, microbial and guar galactomannan (Fig. 4A, 4B and 4C, respectively). EDAX elemental analysis showed that commercial and microbial glactomannan contained mostly from C, O, N, P, Na, K, Ca, Fe, Zn and Se elements. However, guar extracted glactomannan contained from C, O, N, P, Na, Mg, Ca, Fe and Se elements with different percentages recorded in Table 1. There is no uncertainty that, all these elements are very essential in human biological activity and human nutrition. They are required for more than three hundred biochemical reactions in our body for example (sustain normal nerve, muscle function, supports immune system, retains the heartbeat balanced, bones remain strong, regulate blood glucose levels and energy and protein production).

A



B



C

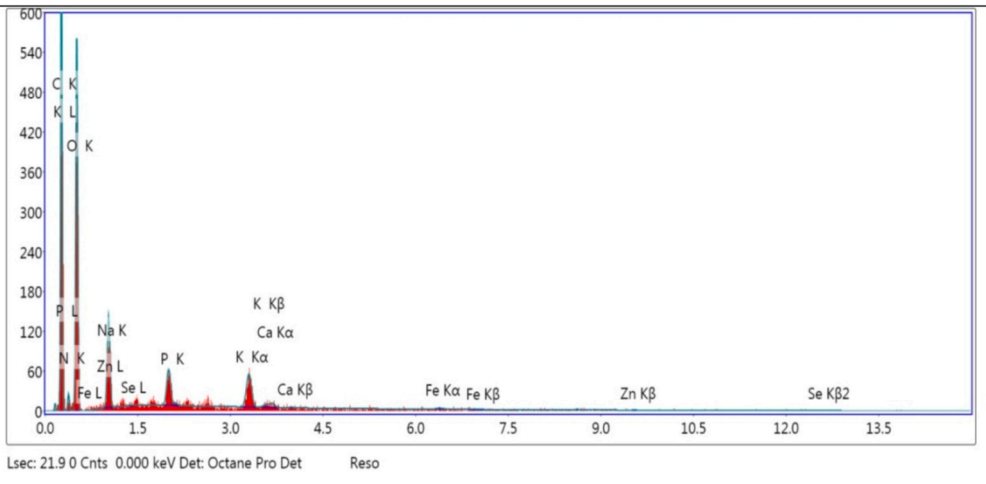


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Fig. 4. EDAX analysisofcommercial (A), microbial (B) and guar (C) galactomannan.

3.6. Prebiotic activities

Commercial, guar and microbial galactomannan samples were examined for their prebiotic activities towards the probiotics *L. casei, L. helveticus* and *L. reuteri.* The prebiotic indices were calculated on the percentage between the growth intensities of the probiotics and the growth intensity of *E.coli* (grown on EPS). Table 2 indicated that microbial galactomannan characterized by their higher prebiotic index towards *L. helveticus* (4.12), *L. casei* (3.84) and *L. Reuteri* (3.36); comparable to the index exhibited slightly similar by guar and commercial galactomannan towards *L. helveticus* (1.35 and 1.70), *L. casei* (2.29 and 2.56) and *L. reuteri* (3.07 and 3.43) as shown in Table 2. As previously detected, that the three galactomannan samples, particularly the microbial source more susceptible to attack by the enzyme system of all probiotic bacterial than caused by *E.coli* attack.

3.7. Yoghurt chemical properties

Titratable acidity of yoghurt treatments increased significantly ($p \leq 0.05$) by increasing the rate of adding the different types of

Table 1
The element of commercial (A), microbial (B) and guar (C) galactomannan.

Element	Weight %			Atomic %			Net Int.			Error %		
	A	B	C	A	B	C	A	B	C	A	B	C
C	45.71	45.86	42.21	52.97	53.07	50	83.66	161.82	106.46	7.66	6.77	8.07
N	2.49	2.47	5.65	2.47	2.45	5.74	1.04	2.01	3.86	45.12	26.73	24.25
O	50.95	50.57	45.93	44.32	43.93	40.85	77.54	150.19	104.8	11.62	10.63	11.36
Na	0.01	0.53	4.37	0.01	0.32	2.7	0.05	3.84	26.69	85.95	22.53	12.62
P	0.07	0.17	0.74	0.03	0.07	0.34	1.2	5.6	19.71	72.19	21.95	12.75
Mg	–	0.17	–	–	0.1	–	–	2.64	–	–	27.63	–
K	0.2	0.05	0.77	0.07	0.02	0.28	3.82	1.84	22.53	33.45	65.02	12.2
Ca	0.08	0.07	0.12	0.03	0.02	0.04	1.33	2.17	3.21	70.05	63.77	63.06
Fe	0.13	0.04	0.08	0.03	0.01	0.02	1.29	0.72	1.25	69.51	72.7	69.97
Zn	0.14	–	0.07	0.03	–	0.02	0.82	–	0.68	73.89	–	76.46
Se	0.22	0.09	0.06	0.04	0.02	0.01	0.55	0.42	0.23	78.67	78.31	93.23

Table 2
The prebiotic index (I) of commercial, microbial and guar galactomannan.

Galactomannan	Prebiotic index (I)		
	L. reuteri	*L. helveticus*	*L. casei*
Commercial	3.07 ± 0.09	1.35 ± 0.02	2.29 ± 0.05
Guar	3.43 ± 0.06	1.70 ± 0.04	2.56 ± 0.06
Microbial	3.36 ± 0.08	4.12 ± 0.1	3.84 ± 0.07

Table 3
The effect of fortifying yoghurt with three types of galactomannan on titratable acidity and pH values during refrigerated storage (6 °C ± 1).

Treatments◘	Storage period (Days)									
	Titratable acidity (%)					pH value				
	0	3	6	9	12	0	3	6	9	12
C*	$0.87^{Ce●}$	0.91^{Cd}	0.9^{Cc}	1.05^{Cb}	1.22^{Ca}	4.64^{Aa}	4.52^{Ab}	4.45^{Ac}	4.36^{Ad}	4.27^{Ae}
T_1	0.93^{Be}	1.02^{Bd}	1.07^{Bc}	1.19^{Bb}	1.27^{Ba}	4.57^{Ba}	4.46^{Bb}	4.40^{Bc}	4.31^{Bd}	4.23^{Be}
T_2	0.96^{Ae}	1.08^{Ad}	1.15^{Ac}	1.23^{Ab}	1.31^{Aa}	4.51^{Ca}	4.39^{Cb}	4.33^{Cc}	4.25^{Cd}	4.17^{Ce}
T_3	0.92^{Be}	1.00^{Bd}	1.07^{Bc}	1.16^{Bb}	1.24^{Ba}	4.56^{Ba}	4.45^{Bb}	4.39^{Bc}	4.32^{Bd}	4.21^{Be}
T_4	0.95^{Ae}	1.01^{Ad}	1.07^{Ac}	1.16^{Ab}	1.29^{Aa}	4.52^{Ca}	4.40^{Cb}	4.33^{Cc}	4.26^{Cd}	4.15^{Ce}
T_5	0.93^{Be}	1.01^{Bd}	1.08^{Bc}	1.17^{Bb}	1.28^{Ba}	4.56^{Ba}	4.44^{Bb}	4.38^{Bc}	4.29^{Bd}	4.20^{Be}
T_6	0.95^{Ae}	0.96^{Ad}	1.07^{Ac}	1.17^{Ab}	1.29^{Aa}	4.53^{Ce}	4.46^{Cb}	4.40^{Cc}	4.31^{Cd}	4.21^{Ce}

C: Yoghurt treatment made with adding 1.5% non-fat dry milk.
T_1 and T_2:Yoghurttreatments fortified with 0.15 and 0.25% of commercial galactomannan respectively.
T_3 and T_4: Yoghurt treatments fortified with 0.15 and 0.25% of guar galactomannan respectively.
T_5 and T_6: Yoghurt treatments fortified with 0.15 and 0.25% of microbial galactomannan respectively.
● A, B, …: The means with the different capital letters within the same column are significantly different ($p \leq 0.05$) and the similar capital letters within the same column are not significantly different ($p > 0.05$).
a, b, …: The means with the different small letters within the same row and treatment are significantly different ($p \leq 0.05$) and the means with the similar small letter within the same row and treatment are not significantly different ($p > 0.05$).

Table 4
The effect of fortifying yoghurt with three types of galactomannan on total solids and fat content (%) during refrigerated storage (6 °C ± 1).

Treatments	Total solids content (%) (days)					Fat content (%) (days)				
	0	3	6	9	12	0	4	8	10	12
C*	12.58 ^{Ba}	12.54 ^{Ba}	12.56 ^{Ba}	12.59 ^{Ba}	12.57 ^{Ba}	3.0 ^{Aa}	3.1 ^{Aa}	3.0 ^{Aa}	3.2 ^{Aa}	3.1 ^{Aa}
T_1	12.77 ^{Ba}	12.75 ^{Ba}	12.73 ^{Ba}	12.70 ^{Ba}	12.75 ^{Ba}	3.1 ^{Aa}	3.1 ^{Aa}	3.2 ^{Aa}	3.1 ^{Aa}	3.0 ^{Aa}
T_2	13.26 ^{Aa}	13.31 ^{Aa}	13.32 ^{Aa}	13.31 ^{Aa}	13.27 ^{Aa}	3.1 ^{Aa}	3.0 ^{Aa}	3.1 ^{Aa}	3.1 ^{Aa}	3.2 ^{Aa}
T_3	12.69 ^{Ba}	12.65 ^{Ba}	12.67 ^{Ba}	12.70 ^{Ba}	12.68 ^{Ba}	2.9 ^{Aa}	3.2 ^{Aa}	3.0 ^{Aa}	3.0 ^{Aa}	3.0 ^{Aa}
T_4	13.21 ^{Aa}	13.26 ^{Aa}	13.27 ^{Aa}	13.23 ^{Aa}	13.20 ^{Aa}	3.2 ^{Aa}	3.1 ^{Aa}	3.1 ^{Aa}	3.1 ^{Aa}	3.2 ^{Aa}
T_5	12.72 ^{Ba}	12.70 ^{Ba}	12.68 ^{Ba}	12.63 ^{Ba}	12.69 ^{Ba}	3.1 ^{Aa}	3.0 ^{Aa}	3.1 ^{Aa}	3.2 ^{Aa}	3.0 ^{Aa}
T_6	13.29 ^{Aa}	13.34 ^{Aa}	13.35 ^{Aa}	13.31 ^{Aa}	13.38 ^{Aa}	3.0 ^{Aa}	2.9 ^{Aa}	3.0 ^{Aa}	3.1 ^{Aa}	3.1 ^{Aa}

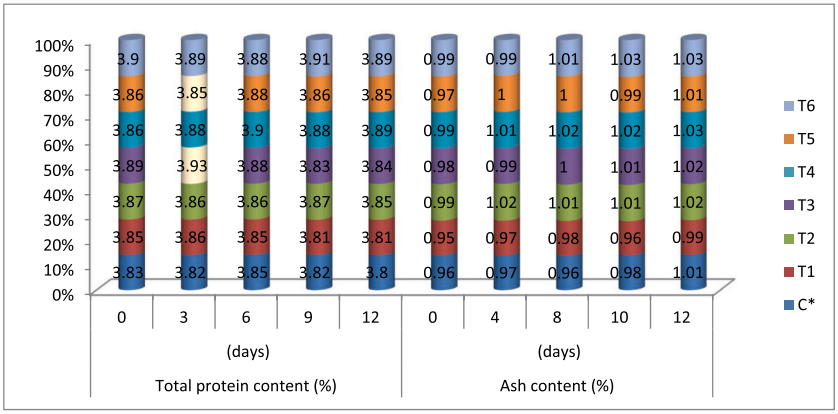

Fig. 5. The effect of fortifying yoghurt with three types of galactomannanon total protein and ash contents (%) during refrigerated storage (6 °C ± 1).

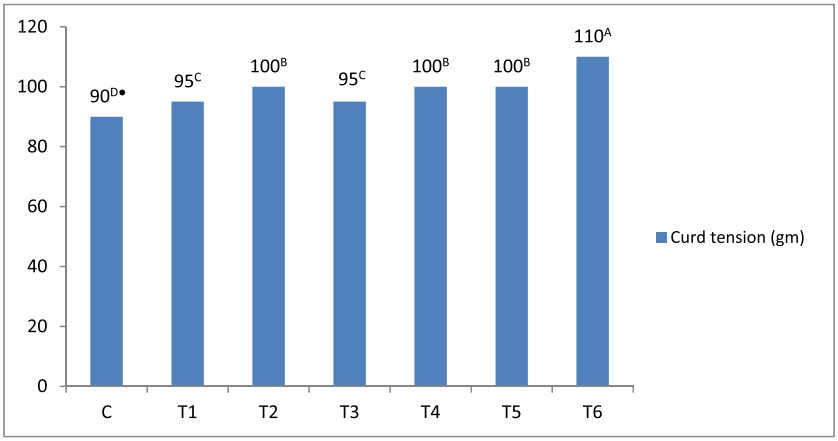

Fig. 6. The effect of fortifying yoghurt with three types of galactomannan on curd tension during refrigerated storage (6 °C ± 1).

galactomannan (Table 3), this might due to the high carbohydrate content of each type of galactomannan. These results were agreed with those recorded by Gibson and Roberfroid [36], and Ahmadi et al. [10]. On the other hand, titratable acidity of all yoghurt treatments increased gradually ($p \leq 0.05$) as storage at low temperature period progressed (Table 3). These results were in agreement with those of Hamed et al. [37] and Kebaryetal [38]. Changes in pH values of yoghurt treatments were shown in (Table 3). pH values decreased significantly ($p \leq 0.05$) by increasing the rate of supplementation. This decrease might be due to the stimulating effect of galactomannan on the growth and activity of yoghurt starter [39,40]. pH values of yoghurt treatments decreased gradually ($p \leq 0.05$) as the storage period progressed (Table 3). These results are in agreement with those reported by Kebary and Hussein [29], Badawi et al. [41] and Kebary et al. [42].

Table 4 showed that there was significant ($p \leq 0.05$) proliferation in total solids content of yoghurt treatments at the rate of adding

Table 5
The effect of fortifying yoghurt with three types of galactomannan on whey syneresis of yoghurt treatments during refrigerated storage (6 °C ± 1).

Treatments[a]	Syneresis (mg whey/100 gm)				
	Storage period (days)				
	0	3	6	9	12
C[a]	$42^{Aa●}$	38^{Ab}	34^{Ae}	36^{Ad}	38^{Ac}
T_1	38^{Ba}	36^{Bb}	32^{Be}	34^{Bd}	38^{Bc}
T_2	34^{Ca}	32^{Cb}	27^{Ce}	30^{Cd}	31^{Cc}
T_3	37^{Ba}	34^{Bb}	29^{Be}	32^{Bd}	36^{Bc}
T_4	33^{Ca}	30^{Cb}	25^{Ce}	28^{Cd}	31^{Cc}
T_5	38^{Ba}	36^{Bb}	30^{Be}	31^{Bd}	33^{Bc}
T_6	34^{Ca}	32^{Cb}	25^{Ce}	26^{Cd}	29^{Cc}

[a] , ● See Table (1).

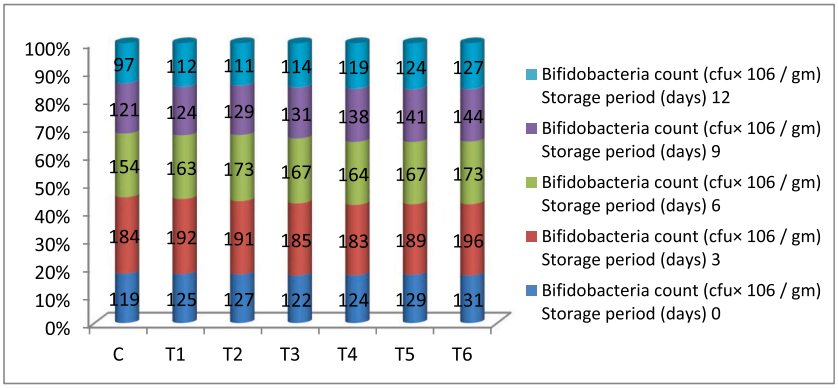

Fig. 7. The effect of fortifying yoghurt with three types of galactomannan on bifidobacteria count (cfu × 10^6/gm) of yoghurt treatments during refrigerated storage (6 °C ± 1).

galactomannan increased. While, there were no significant ($p > 0.05$) differences in fat contents of yoghurt treatments. Total solids and fat content of all yoghurt treatments did not change significantly ($p > 0.05$) during the storage period (Fig. 5). These results were established with those reported by Farag et al. [43], Hamed et al. [36] and Kebary et al. [44]. Ash and protein content of yoghurt treatments were not significantly ($p > 0.05$) different from that of control treatment (Fig. 4). Ash and protein content of all yoghurt treatments did not change significantly ($p > 0.05$) as storage period proceeded [41,42,45].

C: Yoghurt treatment made by adding 1.5% non-fat dry milk.

T_1 and T_2: Yoghurt treatments fortified with 0.15 and 0.25% of commercial galactomannan respectively.

T_3 and T_4: Yoghurt treatments fortified with 0.15 and 0.25% of guar galactomannan respectively.

T_5 and T_6: Yoghurt treatments fortified with 0.15 and 0.25% of microbial galactomannan respectively.

Golden colour means significantly different ($p \leq 0.05$).

3.8. Rheological analysis

Yoghurt curd tension results were presented in Figure (6). Fortifying yoghurt treatments of each type of galactomannan increased the curd tension of the resultant yoghurt treatments. Curd tension increased by increasing the rate of supplementation. Yoghurt was made with microbial galactomannan (T_6) exhibited higher curd tension due to the holes in its morphological structure than corresponding yoghurt treatments those made by adding the other two types of galactomannan while the control yoghurt treatment (C) exhibited the lowest curd tension value.

Table 5 showed the effect of fortifying yoghurt treatments with three types of galactomannan on whey syneresis. Syneresis from all yoghurt treatments decreased gradually ($p \leq 0.05$) as the storage period progressed and reached their minimum values on the 6th day of storage period, then increased up to the end of storage period (Table 5). These results are in agreement with those reported by Farooq and Haque [46], Kebary and Hussein [29] and Kebary et al. [42]. Yoghurt treatment (T6) exhibited the lowest whey syneresis value while the control treatment exhibited the highest whey syneresis value. Fortifying yoghurt treatments with the three types of galactomannan caused a significant ($p \leq 0.05$) reduction of whey syneresis (Table 5). There was a negative correlation between the rate of supplementation and whey syneresis. These results might be due to the high water holding capacity of the three types of galactomannan, the similar results were reported by Lal et al. [13].

Table 6
The effect of fortifying yoghurt with three types of galactomannan on organoleptic properties of yoghurt treatments during refrigerated storage (6 °C ± 1).

Treatment*	Flavour (45)					Body and texture (35)					Appearance (10)				
	Yoghurt samples (days)														
	0	3	6	9	12	0	3	6	9	12	0	3	6	9	12
C	40^{ABa}●	39^{ABa}	39^{ABab}	36^{ABb}	36^{ABc}	25^{Da}	26^{Da}	25^{Dab}	24^{Db}	23^{Dc}	6^{CDa}	6^{CDa}	6^{CDa}	5^{CDb}	4^{CDc}
T_1	41^{Aa}	41^{Aa}	40^{Aab}	38^{Ab}	35^{Ac}	28^{Ca}	28^{Ca}	27^{Cab}	25^{Cb}	24^{Cc}	7^{Ca}	7^{Ca}	6^{Ca}	6^{Cb}	5^{Cc}
T_2	42^{Aa}	41^{Aa}	39^{Aab}	37^{Ab}	35^{Ac}	30^{Ba}	30^{Ba}	29^{Bab}	27^{Bb}	26^{Bc}	8^{ABa}	8^{ABa}	7^{ABa}	6^{ABb}	6^{ABc}
T_3	41^{Aa}	40^{Aa}	39^{Aab}	37^{Ab}	35^{Ac}	27^{Ca}	26^{Ca}	26^{Cab}	25^{Cb}	24^{Cc}	7^{Ca}	7^{Ca}	6^{Ca}	6^{Cb}	5^{Cc}
T_4	42^{Aa}	42^{Aa}	40^{Aab}	38^{Ab}	36^{Ac}	31^{Ba}	31^{Ba}	30^{Bab}	29^{Bb}	27^{Bc}	8^{ABa}	8^{ABa}	8^{ABa}	7^{ABb}	6^{ABc}
T_5	42^{Aa}	41^{Aa}	40^{Aab}	39^{Ab}	36^{Ac}	30^{Ba}	31^{Ba}	30^{Bab}	29^{Bb}	27^{Bc}	9^{ABa}	8^{ABa}	7^{ABa}	6^{ABb}	6^{ABc}
T_6	43^{Aa}	42^{Aa}	40^{Aab}	38^{Ab}	36^{Ac}	33^{Aa}	33^{Aa}	31^{Aab}	29^{Ab}	29^{Ac}	9^{Aa}	9^{Aa}	9^{Aa}	8^{Ab}	8^{Ac}

Table 7
The effect of fortifying yoghurt with three types of galactomannan on organoleptic properties of yoghurt treatments during refrigerated storage (6 °C ± 1).

Treatment*	Acidity (10)					Total score (100)				
	Yoghurt samples (days)									
	0	3	6	9	12	0	3	6	9	12
C	8^{Aa}	7^{Aab}	7^{Abc}	6^{Ac}	5^{Ad}	79^{Da}	78^{Da}	77^{Dab}	71^{Db}	68^{Dc}
T_1	9^{Aa}	9^{Aab}	7^{Abc}	8^{Ac}	7^{Ad}	85^{Ca}	85^{Ca}	80^{Cab}	77^{Cb}	71^{Cc}
T_2	9^{Aa}	8^{Aab}	8^{Abc}	6^{Ac}	6^{Ad}	89^{Ba}	87^{Ba}	83^{Bab}	76^{Bb}	73^{Bc}
T_3	8^{Aa}	8^{Aab}	7^{Abc}	6^{Ac}	5^{Ad}	83^{Ca}	81^{Ca}	78^{Cab}	74^{Cb}	69^{Cc}
T_4	9^{Aa}	7^{Aab}	7^{Abc}	6^{Ac}	5^{Ad}	90^{Ba}	88^{Ba}	85^{Bab}	80^{Bb}	74^{Bc}
T_5	8^{Aa}	7^{Aab}	6^{Abc}	5^{Ac}	5^{Ad}	89^{Ba}	87^{Ba}	83^{Bab}	79^{Bb}	74^{Bc}
T_6	9^{Aa}	9^{Aab}	8^{Abc}	8^{Ac}	6^{Ad}	94^{Aa}	93^{Aa}	89^{Aab}	85^{Ab}	79^{Ac}

Yoghurt treatment T_6 that made by adding 0.25% of microbial galactomannan was the most acceptable yoghurt treatment and gained the highest total scores followed by T_2, T_4 andT_5.Organoleptic scores of all yoghurt treatments did not change significantly ($p > 0.05$) during the first six days of refrigerated storage period then decreased as storage period progressed [38, 44, 47, 48]. It is possible to fortify the cow's milk with 1.5% NFDM and 0.25% of microbial galactomannan to make a good quality yoghurt.

3.9. Microbiological analysis

Figure (7) showed the counts of bifidobacteria during the cold storage of yoghurt treatments. Counts of bifidobacteria increased up to the 3rd day of storage period then declined till the end of storage period. These results might be due to the effect of cold storage and acidity development on the rate of bacterial growth. These results are in agreement with those obtained by Kebary et al. [47], Badawi et al. [41] and Kebary et al. [42]. These results revealed that even after storage for 12 days yoghurt treatments contained counts of bifidobacteria higher than those should be present to achieve their health benefits. There was obvious increasing in the counts of bifidobacteria in yoghurt treatments made with adding galactomannan comparing with control treatment which might be due to higher carbohydrate content of galactomannan which stimulate the growth of bifidobacteria.

3.10. Sensory evaluation

Tables (6) and (7) showed scores of organoleptic properties (flavor, body & texture, appearance, acidity and total scores) of yoghurt treatments. There were no significant ($p > 0.05$) differences between yoghurt treatments in the scores of flavor and acidity. These results indicated that fortifying yoghurt treatments with three types of galactomannan caused a significant ($p \leq 0.05$) improvement of body & texture, appearance and the total scores of the resultant yoghurt treatments. The increase in scores of body &texture, appearance and total scores were proportional to the rate of supplementation.

4. Conclusion

There is an increased interest in glactomannan and its application, particularly in the yogurt food industries.Our study focused on producing different types of glactomannan (chemically and microbial extracted). Optimize the extraction method to minimize contamination of galactomannan. Therefore, characterizing them in comparison with the commercial using different analytical tools to understanding the chemistry of galactomannan. Galactomannans as food additives were exhibited potent physical, chemical, microbial, sensory evaluation and prebiotic activity in yogurt industry. Rheological analysis showed that microbial galactomannan was most acceptable yogurt treatment.

Author contribution statement

Tamer I. M. Ragab, Al Shimaa Gamal Shalaby: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Khadega R. M. Badawi: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mohamed Ahmed Naeem: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Wafaa A. Helmy: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

No data was used for the research described in the article.

Declaration of competing interest

there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e17330.

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