

EFFECT OF CAROB GALACTOMANNANS ON STARTER CULTURES VIABILITY AND ON TEXTURAL PROPERTIES OF REFRIGERATED NON-FAT YOGHURT

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ABSTRACT. Low-fat and non-fat yoghurts have some rheological changes particularly in syneresis and viscosity. Hydrocolloids such as galactomannans (locust bean gum: LBG) can act as fat replacers and can improve the rheological properties of low and non-fat yoghurts. The present study investigates the effect of carob galactomannans on both the viability of starter cultures and textural properties of non-fat yoghurt during cold storage period (4 °C). Carob galactomannans were extracted from carob seeds. Yoghurt was made from skimmed milk powder with the addition of starter culture and locust bean gum at different concentrations (0.01, 0.02, 0.05 and 0.1% W/V). The yoghurt was stored for 28 days at 4°C. Changes in starter microorganisms' viability, pH, viscosity and syneresis were measured after 24h of manufacturing than every 7 days. Results showed that starter culture microorganisms (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) showed better (P<0.05) retention of viability in yoghurt supplemented with galactomannans (0.05 and 0.1%) in comparison with control. LBG did not showed a significant (P<0.05) effect on pH changes during refrigerated storage of yoghurt. However its hydrocolloid improved (P<0.05) the viscosity and prevented the defect of syneresis during storage at 4°C. Additionally, panelists preferred yoghurt sample with 0.05% LBG. This suggests that these polysaccharides can be used as fat replacers to reduce the problems associated with elimination of fat in fermented dairy products.

Keywords: *Ceratonia celiqua* L., galactomannans, non-fat-yoghurt, texture, viability.

INTRODUCTION

In recent years, consumer's demand for low and free fat food products has increased due to increasing awareness of the harmful impacts of fat on consumer's health [1]. Consumption of non fat dairy products is associated with lower risk of cardiovascular and inflammatory diseases, colon cancer and atherosclerosis as well as fulfill important nutrients requirements for humans [2]. Despite these benefits, it has been reported that low-fat yoghurt has some rheological defects, such as a weak texture and whey separation [3]. Furthermore, these products have a different texture than that of full-fat dairy products [4].

To obtain low fat dairy product with good texture and stability, dietary polysaccharides namely pectin, carrageenans and galactomannans are added to yoghurt [5]. These act as stabilizer, thickener and gellifier agents increasing consistency and preventing whey separation [6]. The carob galactomannans is a yellowish powder made from grinding the

seed endosperms of carob fruit (*Ceratonia siliqua* L.). Galactomannanes are neutral hydrocolloids which consist in linear chains of β -1-4 linked mannose units with α -1-6 linked galactose units. At low concentration they form viscous solutions unaffected by pH, ions or heat treatment [7]. They are characterized by their large functional properties and their low cost [8].

Carob galactomannans are classified by the European Codex as accepted human food additive (E410) and are widely used in the food industry [9]. Several studies have been done to improve the textural properties of low-fat and non-fat yoghurt using an association of hydrocolloids [7]. However, there is no data on the effect of carob galactomannan on the survival of starter cultures during cold storage. The present work aims to investigate the effect of carob galactomannan on starter culture viability, viscosity, syneresis and texture of non-fat yoghurt stored at 4°C during 28 days.

MATERIALS AND METHODS

Carob seeds

Mature pods of *Ceratonia siliqua* L. were collected from trees growing naturally in Algeria (Sidi Lakhder, Mostaganm). The seeds were manually separated and stored at room temperature until galactomannans were extracted.

Skim milk powder

Spray dried skimmed milk powder (0% fat) was obtained from Belgomilk CVBA-Belgium.

Yoghurt starter culture

Pure commercial starters freeze-dried cultures *Streptococcus thermophilus* and *Lactobacillus delbruekii ssp bulgaricus* (YF-L811) were obtained from Hansen CHR (Denmark). Inoculum was prepared by dispersing 100 mg of dried starter culture in 50 mL of autoclaved skimmed milk (10g.100mL⁻¹ W/V). The pre-culture was used to inoculate the skim milk after it had been blended and activated at 42° C for 30 min. The enumeration of these pre-cultures was about 6.5 Log CFU.mL⁻¹.

Extraction and purification of carob seed galactomannans

For one hour, whole carob seed (100 g) were added to 800 mL of boiling water (100 °C), causing the seeds swell but not rupture the tegument. The seeds were washed, and then they were manually separated to their three constituents (tegument, germ and endosperm). The endosperms were dried for 1-2 hours at 100 °C, milled and sifted to obtain LBG (locust bean gum) flour [10].

Obtained powder (1.3 g) was gradually added to 100 mL of distilled water. This solution was stirred for 1h then heated for 30 min at 80°C while stirring. The resulting dispersion was cooled at room temperature then centrifuged at 28000 g for 1 h, and then the supernatant was recovered.

The solubilized galactomannans were precipitated by isopropanol. Filtration was used to collect the precipitated galactomannans which were then washed twice with acetone and diethyl ether. The nit was dried at 30 °C, and crushed to fine powder [11, 12].

Yoghurt making

skimmed milk and galactomannans solutions were prepared separately according to the method described by Unal et al. [1].

The skim milk was reconstituted at 20 g.100 mL⁻¹ and tentalised by steaming three days in a row at 85 °C for 30 min.

LBG stock dispersions of 0.02, 0.04, 0.1 and 0.2 g.100 mL⁻¹ were made by dissolving gum powder in distilled water heated at 80°C. The solutions were stirred for 2 h then cooled at room temperature and stored at 5°C. Reconstituted milk and hydrocolloids solutions were added with distilled water at the end of stirring to compensate volume of water loosed during evaporation.

Mixture of skimmed milk and galactomannans were obtained by adding equal volumes of reconstituted milk and various galactomannans solutions, which were then heated at 80 °C for 30 min. The final mixtures contained 10 g.100.mL⁻¹ skimmed milk and 0.01, 0.02, 0.05 or 0.1 g 100.mL⁻¹ galactomannans [1].

All milk samples were heated to 85°C and held there for 30 min before being cooled to approximately 45 °C, and inoculated with 3% (V/V) of pre-culture *Streptococcus thermophilus* and *Lactobacillus bulgaricus* at equal cell numbers (1 :1). The mixtures were thoroughly mixed and dispersed into 50 mL polystyrene cups, and incubated at 42°C until the pH reached to 4.7±0.2. The fermented milk was cooled at 4±1 °C, and the samples were analyzed after 1, 7, 14, 21, and 28 days of cold storage [1, 3].

Analysis

Post-acidification

After the milk had been fermented, the changes in the yoghurts during storage were determined after 1st day (D1), 7th days (D7), 14th days (D14), 21st days (D21) and 28th days (D28) of cold storage at 4°C by measuring the pH using a digital pH-meter (pH 211 Microprocessor, HANNA instruments, France).

Viscosity

The viscosity values of yoghurt was determined after 1st day (D1), 7th days (D7), 14th days (D14), 21st days (D21) and 28th days (D28) of cold storage at 4°C using a digital viscometer (Brookfield Digital viscometer SNB-1, USA) by stirring yoghurt samples for 40 s at 4 rpm at 25 °C.

Syneresis

The whey separation index was determined by measuring the amount of spontaneously dissociated whey in milliliters (mL) (%:V/V), by direct measurement of dissociated whey versus the yoghurt quantity in the cup [13]. Whey separated from yoghurt was collected from cup titled at a 45° angle for 10 s. The syneresis index expressed in percentage consisting the of the collected whey's weight relative to the initial yoghurt weight [2].

Determination of starter cultures viability in yoghurt stored at 4°C

The survival of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* in yoghurt was determined after 24h then every 7 days of refrigerated storage on a sample of 1 mL

of yoghurt diluted at 1/10 with a sterile solution (0.1% peptone W :V) for counting the colonies obtained after incubation on MRS (de Man Rogosa and sharpe) agar acidified at pH 5.4 and ST (*Streptococcus thermophilus*) agar for *Lactobacillus bulgaricus* and *Streptococcus thermophilus* respectively .

Sensory evaluation

The sensorial properties of different yoghurt samples (taste, flavor, color, aftertaste and appearance) were evaluated by 25 panelists between the ages of 18 and 42 years. Each panelists rated yoghurt samples on a 5-point hedonic scale for each attribute. The sensory evaluation was carried out in a standardized room at 25 °C between 10:00 and 12:00 h.

Statistical analysis

Each experiment was carried out in triplicate. Mean and standard deviations were calculated from these values using Microsoft Excel 2010.

Statistical analysis was performed subjecting the data to analysis of variance (ANOVA) using XLStat Pro (version 7.5, French). Means were subjected to Keul's test and $P < 0.05$ was considered statistically significant

RESULTS AND DISCUSSION

Extraction and purification of locust bean gum

The carob seed consists of husk, endosperm and germ, all of which are protected by a very tight unbroken envelope [14]. Heat treatment of seeds at 100°C had lead to an increase in their volume (Fig. 1.a). As a result, the coat was easily broken and manually separated (Fig. 1.b).

The proportion of husk (30%-33%), endosperm (42%-46%) and germ (23%-25%) obtained in this study was of about 31.25%, 40.15% and 28.54%, respectively. These values are in accordance with those reported in previous studies which have demonstrated that the main components of carob seeds are husk (30 to 33%), endosperm (42 to 46%) and germ (23 to 25%) [14].

In this study, crude locust bean gum obtained by grounding endosperms was yellowish. This could be due to the presence of some tannic substances and husk pigment in the crude gum which can determine its functional properties such as viscosity [15, 16]. The purification process was effective in removing all impurities. Unacceptable components were removed from crude gum powder by purification which provides a clear product.



Fig. 1. Different parts of carob seeds.

Post-acidification of yoghurt

Figure 2 depicts pH variation in the refrigerated yogurt samples during 28 days. The initial yogurts pH ranged from 4.51 to 4.90. All yoghurt samples have shown a progressive decrease in pH values during refrigerated storage, with the pH dropping down to 4.33-4.52 after 4 weeks. However, it was noticed that there was no significant difference ($P < 0.05$) between pH decrease in all yoghurt samples. Changes in pH are explained by the yoghurt starter's activity which can produce lactic acid at 4°C as described by Luquet and Corieu [17]. Glusa et al. [18] have reported that *Lactobacillus bulgaricus* can produce lactic acid at 4°C which lead to a post-acidification of the refrigerated products. The role of LBG in preventing post-acidification in yoghurt was not significant ($P < 0.05$) in comparison to the control sample, this can be explained by a production of lactic acid at low temperature in presence or in absence of galactomannans. The study conducted by Ünal et al. [1] the physical properties of low-fat yoghurt affected by storage time, addition of carob galactomannans and milk dry matter showed that the pH of all yoghurt samples decreased during refrigerated storage.

However, the average pH values of yoghurt obtained with 0.02, 0.05 and 0.1% galactomannans addition were found to be lower than yoghurt without (0%) or with 0.01% galactomannans supplementation. On the seventh day of cold storage, the lowest pH value was recorded in yogurts supplemented with locust bean gum (4.66 to 4.40) when compared to the control (4.75). These values decreased during refrigerated period to reach 4.57 to 4.37 on the 14th day of storage and 4.45 to 4.33 on the 28th day of storage. Similar tendencies for pH values were noticed by Sichani et al. [19] for yoghurt containing cress seed gum and locust bean gum during storage.

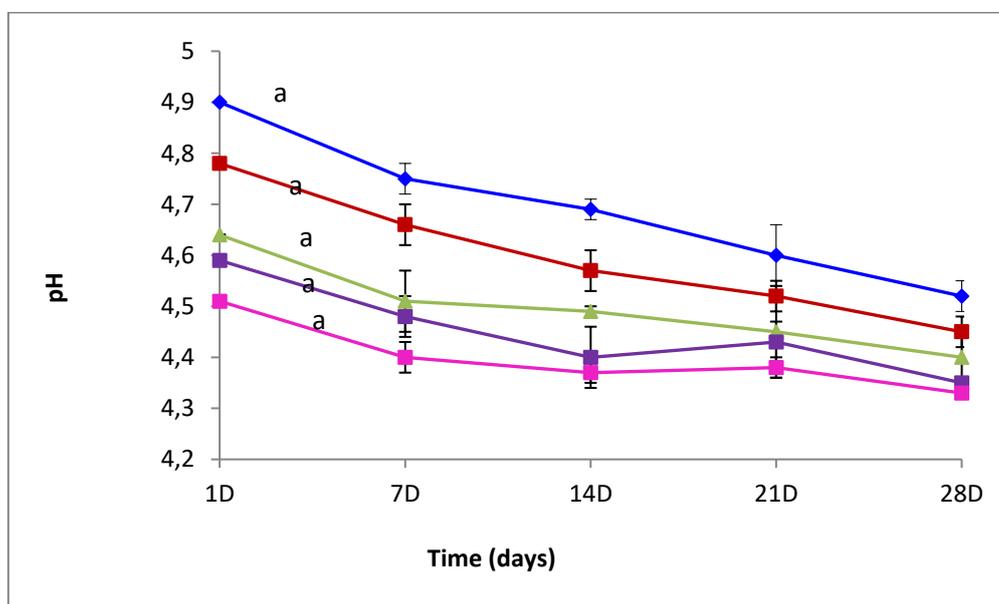


Fig. 2. Changes in pH of non-fat yoghurt without (0% : control) (■) or supplemented with 0.01 (■), 0.02 (▲), 0.05 (■) and 0.1% (◆) of carob galactomannans during 28 days of storage at 4°C. Alphabetic letters indicate no significant difference at $P < 0.05$.

The capacity of production of some basic metabolites by *Streptococcus thermophilus* during storage can explain the increase in the pH values reported on the 21th day of storage in some yoghurt samples as described by Ramchandran and Shah [3].

Viscosity changes

The changes in the viscosity values of yoghurts containing various concentrations of carob galactomannans during refrigerated storage are presented in fig. 3. Obtained results have revealed that yoghurt sample without galactomannans had the lowest viscosity ($P<0.05$), but increasing the LBG level resulted in a higher viscosity. Moreover, statistical analyses have shown a significant ($P<0.05$) increase in yoghurt viscosity in samples added to 0.05 or 0.1% of locust bean gum compared to the control sample.

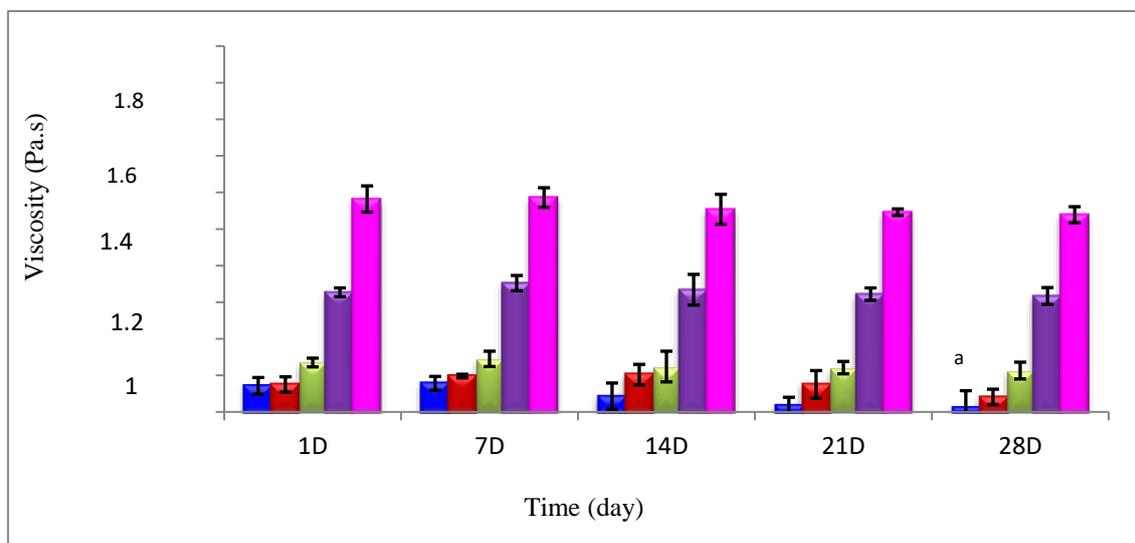


Fig. 3. Viscosity changes of non-fat yoghurt without (0% : control) (■) or supplemented with 0.01% (■), 0.02% (■) 0.05% (■) or 0.1% (■) carob galactomannans during 28 days of storage at 4°C. Alphabetic letters indicate significant difference at $P<0.05$.

In the first day of storage, the viscosity of yoghurt samples supplemented with LBG ranged from 1.088 ± 0.031 to 1.579 ± 0.028 Pa.s compared to 1.078 ± 0.023 Pa.s recorded in the control sample. The viscosities of yoghurts samples with galactomannans were significantly ($P<0.05$) improved, and that of 0.1% LBG was found to have the highest values for all storage periods. The same observations was reported by Hematyar et al. (2012) on xanthan and carrageenan gum who showed that these polysaccharides increased yoghurt viscosity. According to Nautiyal [21], increase in the yoghurt viscosity can be explained by the galactose: mannose series arrangement in galactomannans which are excellent stabilizer of food formulations.

In the present study, it has been noticed that means for viscosity of control sample increased from 1.071 ± 0.023 Pa.S (recorded after 24 h of cold storage) to 1.082 ± 0.019 Pa.S (recorde in the 7th day of storage), however, these changes were not significant ($P<0.05$). Similarly, the changes of viscosity values observed between the 1st and 7th days of cold storage of yoghurt samples supplemented with LBG were not significant ($P<0.05$) (viscosity values of yoghurts ranged from 1.098 ± 0.005 to 1.586 ± 0.027 Pa.S). Similar observations were reported by Abu Jdayil and Mohameed [20] who reported that the

increase in the viscosity during storage can be explained by the protein–protein interactions and protein rearrangement.

However, after 4 weeks of refrigerated storage, the viscosity of the control sample decreased strongly in LBG. This decrease in viscosity could be due to the proteolytic activity of lactic acid bacteria, which continued to hydrolyze proteins decreasing viscosity and rigidity of gel yoghurt [22]. Tamime and Robinson [23] observed that the apparent viscosity of yoghurt decreases during storage time.

Syneresis

Whey separation on the gel is a serious textural defect in yogurt production; serum is accumulated on the surface of the gel resulting to consumer unacceptance of the product [24]. The syneresis values measured in yoghurts samples stored at 4°C shown in table 1.

Table 1. Syneresis values of non-fat yoghurts supplemented with different levels of carob galactomannans during 28 days of storage at 4°C.

LBG addition in yoghurt (%)	Storage period (day)				
	01	07	14	21	28
0	7.66±1.15 ^c	9.00±1.0 ^c	13.00±1.0 ^d	13.33±0.57 ^d	15.33±1.52 ^d
0.01	0±00 ^a	1±00 ^a	4.66±0.57 ^b	7.00±0.9 ^c	9.66±0.57 ^c
0.02	0±00 ^a	0±00 ^a	0±00 ^a	0±00 ^a	2.00±00 ^a
0.05	0±00 ^a	0±00 ^a	0±00 ^a	0±00 ^a	0±00
0.10	0±00 ^a	0±00 ^a	0±00 ^a	0±00 ^a	0±00 ^a

Alphabetic letters indicate significant difference at P<0.05

Yoghurt made without galactomannans have shown higher syneresis values compared to the yoghurts incorporated with LBG. The lowest syneresis was observed in samples containing 0.02, 0.05 and 0.1% galactomannans which can be due to the remarkable water-binding properties of these hydrocolloids. The amount of whey separation measured in the control sample on the first day of storage was 7.66±1.15%, and it increased significantly (P<0.05) throughout the storage period to reach 13.0±1, 13.33±0.57 and 15.33±1.52 after 2, 3 and 4 weeks of cold storage, respectively.

Lucey [25] reports that the post-acidification is one of the factors that can increase the production of whey in yoghurts. However, the whey separation in yoghurt containing 0.01 LBG and stored for 7 days at 4 °C was lower (1% after the first day of storage) compared to the control sample rising to 09.66±0.57% on the 28th day of storage. Although, adding of 0.02 to 0.1% of galacomannans to yoghurt caused significant (P<0.01) decrease in syneresis and whey separation and prevented this fault during the storage period.

According to Hematyar et al. [26], samples of yoghurt with gums had less syneresis during storage period. As a result, these polymers are used in low fat dairy product to enhance the viscosity. When added at an appropriate level, they reduce the syneresis defect and improve the apparent viscosity. Furthermore, Rafik et al. [27] stated that gums regulated the problem of defect of serum separation in dairy products by interaction with water molecules reducing the whey separation on the surface of product.

Lactic acid bacteria viability

The effect of galactomannans on the viable count of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* was determined over 28 days of storage of yoghurt at 4 °C. Figure 4 depicts obtained findings.

The results showed that the viable counts of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in all of experimental yoghurts decreased as storage time elongates. This can be due to the production of some toxic metabolites by lactic acid bacteria such as bacteriocins, acids and H₂O₂.

In the control sample, the viable cells of *Streptococcus thermophilus* decreased from 8.12±0.15 (24h) to 7.18±0.13 and 5.86±0.14 CFU/mL after 2 and 4 weeks of refrigerated storage, respectively. *Lactobacillus bulgaricus* counts decreased from 8.11±0.09 (24h) to 7.04±0.23 and 5.68±0.13 CFU/mL after 2 and 4 weeks of refrigerated storage, respectively.

Reductions of biomass of *Streptococcus thermophilus* recorded in yoghurts supplemented with 0.01, 0.02, 0.05 and 0.1% of LBG after 2 weeks of refrigerated storage were approximately 1.64, 1.13, 1.01 and 0.86 log CFU, respectively, and those recorded after 4 weeks of cold storage were approximately 3.2, 1.91, 1.54 and 1.3 log CFU, respectively. Those of *Lactobacillus bulgaricus* obtained in yoghurts supplemented with 0.01, 0.02, 0.05 and 0.1% of LBG after 2 weeks of refrigerated storage were of about 1.8, 1.42, 1.28 and 1.18 log CFU, respectively, and they reached 3.36, 2.05, 1.67 and 1.55 log CFU, respectively, after 4 weeks of refrigerated storage.

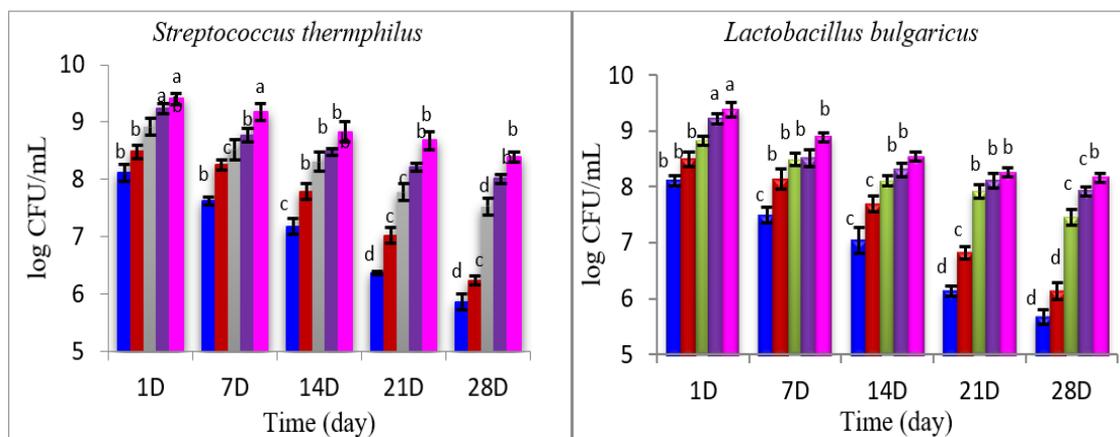


Fig. 4 : Viable count of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in non-fat yoghurts without (0% : control) (■) or supplemented with 0.01% (■), 0.02% (■) 0.05% (■) or 0.1% (■) carob galactomannans during 28 days of storage at 4°C. Alphabetic letters indicate significant difference at $P < 0.05$.

Dave and Shah [28] observed that during 35 days of yoghurts storage, *Lactobacillus bulgaricus* and *Streptococcus thermophilus* showed changes in cells number and decrease gradually. Luquet and Corieue [17] have reported that lactic acid bacteria maintain their activity during the storage of fermented milk at 4 °C and continue to produce organic acids from lactose, which negatively affects their viability. Bruno et al. [29] observed that the lactic acid bacteria count reduced during storage at 4 °C and the reduced amount depended on both, cultures type and carbon source.

The addition of locust bean galactomannans to yoghurt has a significant ($P < 0.05$) influence on the survival of culture strains and they are present at sufficiently high levels during the refrigerated storage period in yoghurts supplemented with these polysaccharides. These results can be explained by the protective effect of galactomannans on lactic acid bacteria against toxic metabolites (acids, bacteriocins etc...). Also, it has been noticed that the viability of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in yoghurt is affected by the concentration of LBG in yoghurt; thus, high levels of galactomannans give better protection of lactic acid bacteria. Indeed, after 4 weeks of cold storage, the viability retention of the starter organisms in yoghurts containing 0.01, 0.02, 0.05, and 0.1% of LBG is about 66.06, 79.76, 83.87 and 86.58%, respectively for Streptococci strain and is about 64.59, 78.17, 82.58 and 84.05% for lactobacilli strain, respectively. As such, yoghurt added with 0.02 to 0.1% (W/V) of LBG contains sufficient number of viable cells starter organisms all over storage period, with no significant differences and starter cultures showed better retention of viability (> 7 log CFU/mL) compared to the sample control and yoghurt added with only 0.01% (W/V) of LBG, which was reduced by approximately 3 log cycle (< 7 log CFU/mL after 28d of refrigerated storage). Furthermore, a minimum of 7 log UFC/g of viable cells of starter culture is required by the food regulations at the consumption time.

In general, our results have shown that starter cultures showed significant ($P < 0.05$) retention of viability (7 log CFU/mL) in the presence of locust bean gum during the refrigerated storage compared to the control sample.

Aspect and Sensory evaluation of yoghurt supplemented with locust bean gum

As shown in fig. 5, the addition of galactomannans to yoghurt improves its texture and firmness compared to the control sample. Galactomannans incorporation in yoghurt had an influence on the improvement of its aspect and texture.

Similar observations have been reported for galatomanans and other gums, Hess et al. [30], Hematyar et al. [26] and Unal et al. [1] suggest that this hydrocolloid enhance the rheological and textural properties of fermented milk. According to Tamime and Robinson [23], hydrocolloids can increase the firmness and viscosity of yoghurt by binding water (restricting the movement) and interacting with milk proteins to stabilize the protein network. Koksoy and Kilic [31] reported that the addition of hydrocolloids in ayran, a yoghurt drink produced in Turkey had a significant effect on taste, odor, consistency and overall acceptability.

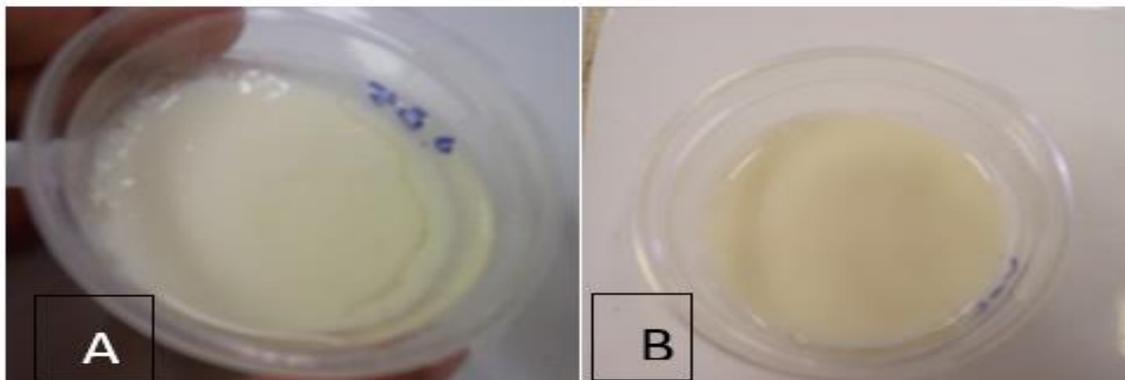


Fig. 5. Visual appearance of non-fat yoghurts supplemented with 0.02% (A) and 0.1% (B) locust bean gum supplementation after 4 weeks of storage at 4°C.

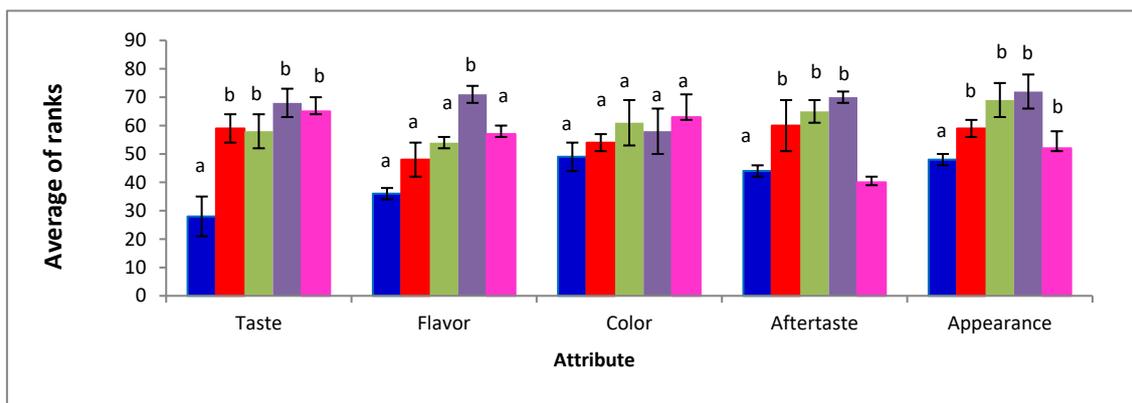


Fig.6. Organoleptic evaluation of non-fat yoghurts without (0%: control) (■) or supplemented with 0.01% (■), 0.02% (■) 0.05% (■) or 0.1% (■) carob galactomannans. Alphabetic letters indicate significant difference at $P < 0.05$.

In the present study, panelists estimated that the taste of yoghurt samples added with 0.01, 0.02 and 0.05% of LBG was better ($P < 0.05$) than both, the control sample and yoghurt supplemented with 0.1% of LBG. Moreover, the panelists have shown a preference ($P < 0.05$) for the flavor of yoghurt supplemented with 0.05% of carob galactomannans comparatively to the other samples. According to the color, no significant difference ($P < 0.05$) among all the yoghurt samples was detected by the panelists, this can be due to the purification of gum which ensure an elimination of all impurities susceptible to be present in the crude gum and it provides a white powder. The panelists detect an aftertaste in the yoghurt supplemented with 0.1% of galactomannans comparatively to the control sample and yoghurt added with 0.01 to 0.05% of galactomannans. In general, panelists have found that addition of 0.05% of carob galactomannans on yoghurt improved significantly ($P < 0.05$) its sensory characteristics.

CONCLUSION

Removal of fat in fermented dairy products induced many textural defects, including syneresis. The rheological properties of low-fat yoghurt may be enhanced after the use of some hydrocolloids as fat replacers such as galactomannans [19]. In this study, the effects of the extracted and purified carob galactomannans on starter cultures viability, pH, viscosity and syneresis of non fat yogurt were evaluated during refrigerated storage. Our findings revealed that LBG had no effect on the pH of low-fat yoghurt during storage; thus, these hydrocolloids improve the viscosity and prevent the defects of syneresis during refrigerated storage.

The addition of carob galactomannans to yoghurt at low concentrations (0.01 to 0.1% W/V) improved the survival of starter cultures during refrigerated storage, and the levels of viable count observed at the end of storage (28 days) exceeded the food regulations ($> 10^6$ CFU.mL⁻¹).

Because of its natural origin, neutral flavor, viscosity and ability to provide odorless products, locust bean gum can be considered as an alternative fat replacer to use in dairy applications due to its ability to reduce syneresis and other textural defaults. Furthermore, it has a beneficial effect on human health.

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Conflict of Interest. “The authors declared that there is no conflict of interest.”

Authorship Contributions. Concept: M.S., H.Z., A.R., Design: M.S., H.Z., A.R., Data Collection or Processing: M.S., Analysis or Interpretation: M.S., H.Z., A.R., Literature Search: M.S., H.Z., A.R., Writing: M.S.

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