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GROWTH BEHAVIOR OF SOME LACTIC ACID BACTERIA IN STIRRED YOGHURT WITH SOME NATURAL SWEETENERS

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ABSTRACT

L. bulgaricus NRRL B-548*, S. thermophilus* NRRL B-512*, L. reuteri* NRRL B-14171 and *L. johnsonii* B-2178 were examined for their growth activity in skim milk containing (7% w/v) six different natural sweeteners (sucrose, glucose, fructose, honey, cane molasses and dibis). The growth activity were monitored by determining the pH and OD at 0, 4, 8, 24 h interval *L. bulgaricus* displayed the highest growth activity throughout the incubation period followed by *S. thermophilus*. While *L. reuteri* and *L. johnsonii* showed the slowest growth activity in skim milk with the natural sweeteners. Regarding the effect of the different sweeteners, dibis and molasse improved the growth and acid production of *L. bulgaricus*. While glucose affected the acid production of *S. thermophilus* and the pH values were the least (4.25) after 24 h of incubation, however no significant differences in the OD value were observed. The different types of sweeteners were not stimulatory or inhibitory to *L. reuteri* or *L. johnsonii*.

There was a decline in the viable counts of all of the examined microorganisms with different rates during the refrigerated storage. However their populations were still above 5 log cycles especially when dibis was used.

Stirred yoghurt was manufactured from sweetened buffaloes' milk*, L. reuteri* or *L. johnsonii* were used as adjunct, the stirred yoghurt was analyzed physiochemically and evaluated organoleptically. Yoghurt manufactured by *L. johnsonii* gained the highest score especially with dibis, molasse or honey.

INTRODUCTION

A greater concern in the use of natural and healthy new substances as food additives and prebiotics have been recently raised (Kneifil and Pacha, 1993). Sucrose and corn syrup have been the traditional and the commonly used sweeteners in the dairy industry . Beside sucrose and corn syrup, honey, cane molasses and dibis (date syrup) could be used as natural sweeteners. All these sweeteners are viewed as value-added since their nutritional value is high (Daviad and Ball 2007, NHB, 1996 and Hayruallah and Irfan 1986.

The beneficiary role of dairy products may be further enhanced by the supplementation of probiotic bacteria. Probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit on the host (Reid *et al.,*2003). Various strains of lactobacilli are considered probiotics, these include *Lactobacillus reuteri* (formely *L . fermentum*) and *Lactobacillus johnsonii* (formely *L. acidophilus*). The former strain beside the general probiotic–associated activities, it can colonize the intestine and vagina and reduce recurrences of bacterial vaginosis, yeast vagintis and urinary tract infections (Reid, 1999; Reid *et al.,* 2001; Reid and Burton, 2002; Cadieux *et al.,* 2002; Reid *et al.,* 2003 and Reid *et al.,* 2004). Although, *L. reuteri* occurs naturally in humans, it is not found in all individuals. Therefore, dietary supplementation is needed to introduce and maintain high levels of *L . reuteri* in some people (Wolf *et al.,* 1995) .

Since milk and dairy products are good-vehicle to deliver probiotic microorganisms to consumers. Therefore, an important aspect in the manufacture of probiotic products is to ensure that bacterial survival retains its effective dosage at the end of shelf life.

Therefore, the objectives of this study were to (i) investigate the ability of probiotic *L. reuteri* , *L. johnsonii* and yoghurt culture to grow in the presence of some natural sweeteners (Honey, Cane molasses, Dibis) in comparison to sucrose, fructose and glucose. (ii) Determine the viability of tested microorganisms during refrigerated storage. (iii) Examine the tested strains for their performance in the manufacture of stirred yoghurt with the previous mentioned sweetners.

MATERIALS AND METHODS

Fresh buffaloes' milk and skim milk were obtained from the Dairy technology unit of Agriculture Faculty Cairo University.

Six sweeteners were used, Cane sugar, Cane molasses, Honey and Date syrup (dibis) were purchased from local market. While fructose 42% and standard glucose were obtained from 10th Ramadan City, National Company for Maize products, Egypt .

Single probiotic strain culture of *L. bulgaricus* NRRL B-548*, S. thermophilus* NRRL B-512*, L. reuteri* NRRL B-14171 and *L. johnsonii* B-2178 were obtained from Northern Regional Research Laboratory, USA.

MRS (Oxoid Manual, 1982) and M_{17} media (Terzaghi and Sandine, 1975) were used for the propagation and enumeration of Lactobacilli and *S. thermophilus* respectively .

Fresh skim milk was divided into equal seven portions. Sucrose, Glucose, Fructose, Honey, Cane molasses and Dibis were added individually at 7% (w/v). Whereas the seventh batch was devoid of added sweeteners and served as control.

All portions were heated at 80ºC/10 min. and cooled to 40ºC. Each portion was divided into 4 aliquots and inoculated with 1% (v/v) *S. thermophilus*, *L. delbrueckii spp bulgaricus, L. johnsonii* and *L. reuteri* and incubated at their optimum temperature for 24 h. The bacterial growth was monitored by determining $O.D_{640}$ and pH at 0,4,8 and 24 h .

The previous incubated samples were stored at 6 ±1 ºC for 21 days and the viability of each organism was assessed at 7 day intervals.

For making of the stirred yoghurt, buffaloes' milk (27 L) was tempered to 45ºC then divided into three portions. Each portion was divided furthermore into six aliquots and sweetened at 7% (w/v) with the different sweeteners. Then the milk was heated to 85ºC/30 min., cooled at 40 - 43 ºC and inoculated with different cultures as follow: The first portionwas inoculated with *L.bulgaricus, S. thermophilus* (1:1) at 2%. The second and the third portions were inoculated by *L. bulgaricus, S. thermophilus* and *L. johnossii* or *L. reuteri* at 1% each respectively. After inoculum with the appropriate inoculum type the mix is incubated at 42ºC until pH reached 4.6 (the time taken to reach 4.6 was recorded for each sample). The curd was cooled and stirred. The stirred yoghurt was distributed to 100 ml plastic retail containers, sealed and stored

at 6 ºC ±1 for 9 days. The samples were subjected to microbiological, physiochemical and sensory analysis at 0 and 9 days.

Bacterial growth was determining by measuring O.D at 640 nm using a spectrophotometer, Mnican 8625 UV/Vis (Desai, Powell and Shah, 2004) . Total viable counts of Lactobacillus strains were determined on MRS agar and *S. thermophilus* was enumerated on M_{17} agar . Acidified MRS agar with pH 5.4 was used for enumeration with Lactobacilli, the plates were overlayed with the same medium. Plates were incubated at 37ºC for 48h (Vanderzant and Slittstoesser, 1992). All viable counts were preformed in duplicate .

Samples were analysed for titratable acidity (T.A%) according to Ling (1963), while pH measurements were carried out using a laboratory pH meter type (3305) JenWay Co., USA. Finally, viscosity was measured using a Brookfield DV II^+ Viscometer equipped with a T-spindle No.3 at 50 rpm and 18.2ºC, and expressed in cPs .

Stirred yoghurt samples were judged by the staff members of the Dairy Sci. department, Faculty of Agric., Cairo Univ.

The values of all experiments were presented as the mean of triplicate analysis. Statistical analysis for obtained data was carried out using the Statistical Analysis System (SAS, 1997) .

RESULTS AND DISCUSSION

The growth behavior of yoghurt culture, *L. reuteri* and *L. johnsonii* in sweetened skim milk was demonstrated by measuring pH and OD (Tables 1, 2, 3, 4). All organisms resulted in a further decrease in the pH and increase in OD values with prolonging the incubation period to 24 h. The rate of changes in these values was the highest with *L. bulgaricus* as it grew faster in milk and reached its maximum level after 24 h., followed by *S. thermophilus*, while both *L. reuteri* and *L. johnsonii* showed a slow growth rate as the pH reached (4.81, 5.50), respectively after 24 h. These results are in accordance with Lazlo avonts *et al*. (2004) and Elli *et al*., (1994), who reported that *L. johnsonii* La l was unable to grow to high cell numbers in milk and thus is due to the low content of free amino acid and small peptides. Also Hidle *et al*. (2003) reported that the poor ability of milk acidification, observed in *L. reuteri* might be due to a week proteolytic activity.

Regarding the effect of different sweeteners type on the activity of the four organisms used, molasses and dibis supported the growth and the acid production of *L. bulgaricus* as they have similar pH (3.8) and OD (1.975 – 1.936 respectively) values after 24 h of incubation. While the lowest OD values was observed with control as well as fructose, being 1.805 and 1800 respectively.

In the case of *S. thermophilus*, glucose showed the least rate of acid production as the pH was (4.25) after 24 h of incubation, However at this period, there was no significant differences in the OD values between the different types of sweetners. So it could be concluded that *S. thermophilus* was not influenced by the sweetener type.

Concerning *L. reuteri* and *L. johnsonii*, It is noticeable that the sweeteners were nor stimulatory or inhibitory as there was no significant

differences between the control and the different sweeteners used. These results are in agreement with Riazi and Ziar (2008), who reported that lactic acid production was not influenced by sweetener type, also partly in agreement with Chick *et al.* (2001) who reported that pasteurized clover honey (70ºC/15 min), sucrose or fructose at the level of 5% (w/w) generates similar improved growth of *S. thermophilus*, *L. bulgaricus* and *L. acidophilus* and that honey did not exert an inhibitory effect at this level. Lustunol and Ghandi (2001) attributed that to the presence of various oligosaccharides presented in honey rather than to its fructose and glucose components which enhanced the acid production of *Bifidobacterium* spp.

Table (1). Effect of some sweeteners on the growth activity of *L. bulgaricus*.

Sweeteners	pH				OD			
	Storage period (hrs)				Storage period (hrs)			
	0	4	8	24	0	4	8	24
Control	6.3	5.91	5.2	3.90	0.106	0.160	0.835	1.805
Sucrose	6.23	5.75	5.12	3.95	0.109	0.170	0.870	1.823
Glucose	6.20	5.73	5.10	3.85	0.11	0.155	0.840	1.849
Fructose	6.23	5.78	5.15	3.85	0.107	0.167	0.892	1.800
Honey	6.13	5.61	5.00	3.93	0.105	0.160	0.835	1.817
Molasses	6.15	5.58	4.90	3.8	0.100	0.179	0.860	1.975
Dibis	6.17	5.6	4.85	3.8	0.101	0.133	0.870	1.936
LSD at 0.05 level for OD after 24 hrs = 0.018								

Table (2). Effect of some sweeteners on the growth activity of *S. thermophilus.*

Sweeteners	pH				OD			
	Storage period (hrs)				Storage period (hrs)			
	0	4	8	24	0	4	8	24
Control	6.20	6.09	5.30	4.1	0.105	0.165	0.84	1.702
Sucrose	6.20	6.00	5.00	4.1	0.106	0.172	0874	1.883
Glucose	6.20	6.00	5.10	4.25	0.108	0.170	0.862	1.867
Fructose	6.20	6.02	5.00	4.1	1.102	0.169	0.8400	1.845
Honey	6.10	5.80	5.10	4.0	0.104	0.168	0.845	1.831
Molasses	6.12	5.09	5.00	4.05	0.109	0.175	0.877	1.893
Dibis	6.15	5.85	5.06	4.1	0.103	0.184	0.881	1.897
LSD at 0.05 level for OD after 24 hrs = 0.295								

Table (3). Effect of some sweeteners on the growth activity of *L. reuteri.*

Sweeteners	pH				OD			
	Storage period (hrs)				Storage period (hrs)			
	0	4	8	24	0	4	8	24
Control	6.25	6.05	5.515	4.81	0.105	0.135	0.220	0.735
Sucrose	6.20	6.00	5.50	4.82	0.103	0.140	0.280	0.700
Glucose	6.22	6.09	5.515	4.85	0.103	0.132	0.280	0.765
Fructose	6.20	6.10	5.05	4.845	0.108	0.135	0.250	0.745
Honey	6.15	5.9	5.52	4.85	0.095	0.1325	0.260	0.740
Molasses	6.12	5.90	5.515	4.84	0.107	0.140	0.275	0.790
Dibis	6.02	5.875	5.55	4.04	0.1035	0.145	0.255	0.770
LSD 0.05								0.055

Table (4). Effect of some sweeteners on the growth activity of *L. johnsonii.*

Sweeteners	pH				OD			
	Storage period (hrs)				Storage period (hrs)			
	0	4	8	24	0	4	8	24
Control	6.2	6.1	5.705	5.50	0.1035	0.128	0.180	0.229
Sucrose	6.26	6.00	5.82	5.55	0.106	0.135	0.195	0.255
Glucose	6.285	5.955	5.765	5.5	0.1035	0.126	0.174	0.240
Fructose	6.225	5.96	5.80	5.5	0.1015	0.134	0.1775	0.259
Honey	6.16	5.98	5.70	5.55	0.1035	0.123	0.170	0.225
Molasses	6.09	5.90	5.70	5.40	0.101	0.140	0.180	0.260
Dibis	6.025	5.94	5.70	5.37	0.1025	0.125	0.1725	0.265
LSD 0.05								0.04

Results in Tables (5,6,7 and 8) showed the viable counts of *L. bulgaricus,* thermophilus, *L.reuteri* and *L. johnsonii* in the sweetened skim milk over 21 days of refrigerated storage. The initial counts of *L. bulgaricus* and *S.thermophilus* was higher than *L.reuteri* or *L. johnsonii* due to the difference in the growth rate of each organism in the previous 24 h of incubation.

In general, at the first week there was no any change in the viable counts of all the microorganisms in the presence of the different types of sweeteners, as the viable counts remained constant with no significant difference when compared with the beginning of storage period. However, the behaviour of each microorganism as affected by the type of sweetener started to be different after the 7th day of storage, where there was a continuous decline in the viable counts of *L. bulgaricus* till the end of storage period when sucrose, fructose, honey, and molasses were added to the skim milk. The decrease in the viable counts was delayed to the end of storage period when dibis and glucose were added.

The rate of the decline of the viable count of *S. thermophilus* ranged between (0.8 log cycle in presence of glucose and 0.37 log cycle in sample sweetened with dibis). Also, the decrease started by the end of first week in the samples contained fructose, honey and molasse while in sucrose, glucose and dibis, the viable counts remained constant till the 14th day of storage.

Almost in all the samples the viable counts in the case of *L. reuteri* remained constant only during the first week, and started to decline till the end of storage period. The decline in the viable count was not affected by the type of sweetener as the decline was ~ 0.7 log cycle.

Similar to *L. reuteri, L. johnsonii* was not affected by the types of sweeteners and the decline in the viable count ranged between (0.54 – 0.64 log cycle) in all samples except dibis, the decline was (0.32 log cycle). Also the decrease in the count were delayed to the end of storage period in the presence of glucose and dibis.

In spite of the decline in the viable counts of all the microorganism during the refrigerated storage, their populations were presented in high levels above 5 log cfu g^{-1} (which satisfyes the criteria for probiotic bacteria and yoghurt cultures). The highest population was observed when dibis is used

being 8.9, 9.00, 6.71, 6.08 log cycle and the lowest were 8.60, 8.54, 5.66 when glucose used and (6.4 log cycle) when fructose was added for *L. bulgaricus, S. thermophilus, L. johnsonii* and *L. reuteri*. These results are in agreement with Schillinger *et al*. (1999), who reported that viability losses between 0 and 3 log units during refrigerated storage of fermented milk and also with Martinez-Villaluenga et al., (2006) who observed a decrease in the viability at 14 days for Lactobacilli and the presence of RFOs (Raffinose family oligosaccharides) enhanced the resistance to refrigeration was ≈ 7 $\log_{10}$ at 21 days of storage in milks with RFOs, and 6.2 $\log_{10}$ viable cell in milks without RFOs.

Table (5). Effect of refrigerated storage period (6 ±1ºC) on the viable count of *L. bulgaricus* .

Sweeteners	Storage period (days)			
	0	7	14	21
	Log cfu g^{-1}			
Control	9.76	9.55	8.95	8.63
Sucrose	9.89	9.60	9.10	8.79
Glucose	9.60	9.55	9.37	8.60
Fructose	9.68	9.43	9.10	8.84
Honey	9.64	9.54	9.05	8.95
Molasse	9.67	9.60	9.15	8.98
Dibis	9.62	9.48	9.62	8.90
L.S.D 0.05	0.27			

Table (6). Effect of refrigerated storage period (6 ±1ºC) on the viable count of *S. thermophilus*.

Sweeteners	Storage period (days)			
	0	7	14	21
	Log cfu g^{-1}			
Control	9.23	9.20	8.80	8.64
Sucrose	9.46	9.60	9.7	9.06
Glucose	9.35	9.20	9.02	8.54
Fructose	9.45	9.25	9.00	8.60
Honey	9.20	9.38	9.00	8.69
Molasse	9.23	9.25	8.90	8.78
Dibis	9.37	9.30	9.25	9.00
L.S.D 0.05	0.24			

Table (7). Effect of refrigerated storage period (6 ±1ºC) on the viable count of *L. reuteri*.

Sweeteners	Storage period (days)			
	0	7	14	21
	Log cfu g^{-1}			
Control	7.20	7.02	7.20	6.50
Sucrose	7.28	7.23	7.09	6.50
Glucose	7.43	7.00	6.84	6.75
Fructose	7.16	7.05	6.50	6.40
Honey	7.32	7.18	6.95	6.62
Molasse	7.41	7.20	7.32	6.72
Dibis	7.38	7.39	7.94	6.71
L.S.D 0.05	0.27			

Table (8). Effect of refrigerated storage period (6 ±1ºC) on the viable count of *L. johnsonii*.

Sweeteners	Storage period (days)			
	0	7	14	21
	Log cfu g^{-1}			
Control	6.45	6.31	6.35	5.78
Sucrose	6.58	6.66	6.84	6.00
Glucose	6.30	6.41	5.64	5.66
Fructose	6.34	6.25	6.03	5.72
Honey	6.30	6.35	6.48	5.76
Molasse	6.45	6.30	5.87	5.83
Dibis	6.40	6.30	6.40	6.08
L.S.D 0.05	0.20			

For the manufacturing of stirred yoghurt, the lactobacilli and the streptococci were enumerated during refrigerated storage. The four used microorganisms behaved similarly in the sweetened yoghurt as in the previous experiment in skim milk and the results were in the same trend and confirmed the previous experiment (data not shown). Since the microorganisms grew well in the sweetened milk during fermentation. And after the 9 days of cold storage the population of these microorganisms remained above 5 log cycle, the minimum level suggested by some authors (Kurmann& Rasic (1991); Samona & Robinson, (1994) for probiotic microorganisms in fermented milks in order to produce therapeutic benefits. However, physicochemical analysis of the stirred yoghurt was shown in Table (9), which displayed the changes in the titratable acidity, viscosity and a sensory evaluation for the samples at zero and after 9 days of refrigerated storage. The results showed that when *L. reuteri* was used as adjunct culture, the development of acidity during the fermentation and the refrigerated storage period was faster than the control and the samples with *L. johnsonii*. This was demonstrated by the reduction of the fermentation time. However, the development of acidity in the samples with or without *L. johnsonii* was similar during the fermentation and storage period. This due to the slow growth rate of *L. johnsonii* in milk. The acidity of the different cultures was affected by the type of sweetener used. When dibis was used, the development of acidity was the highest leading to shortening the incubation period to 5 hours, and it continued till the end of storage period, where the TA% attained it is maximum levels being 0.906, 0.87, 1.11 with yoghurt culture alone, yoghurt culture and *L. reuteri* or *L. johnsonii*, respectively. The different types of sweeteners could be ranged according to their effect on shortening the fermentation period to dibis < honey < fructose < glucose < sucrose and molasse. However, when the development of acidity during the storage period was considered, their order was Dibis > molasses> fructose > honey > sucrose > glucose. The reduction in the total fermentation time and the rapid acid development during the storage period when dibis, molasse and honey might be due to the favorable effect of micronutrients in these sweeteners David (2007) and Khalil, *et al.* (2002).

Concerning the viscosity, data show that samples contain dibis had highest viscosity as it was (385 and 1800) at 0 and 9 days, respectively. These results were in agreement with Simun Zamberline et al., (2007) who reported that the viscosity of stired yoghurt from 0.324 Pa on zero day to

1.912Pa on the 21 days in all samples the viscosity increased during storage this might be attributed to the post acidification which occurs during yoghurt storage, acidity lead to a reinforcement of the strength of the protein net work or the production of exopolysaccharides by microorganisms (Saint-Eve *et al*., 2007) possibly due to increasing hydration (Baizar, Cerning & Desmazed, 1997).

Table (9). Physiochemical and sensory properties of stirred yoghurt produced with different sweeteners.

Sweeteners	Cultures	Fermentation period (hrs)	TA%		Viscosity cps		Sensory (out of10)	
			Storage period, days					
			0	9	0	9	0	9
Sucrose	Y(1)	8	0.745	0.790	220	1492	7	7
	Y+L.J(2)	8	0.770	0.800	250	1500	8	8
	Y+L.R(3)	7	0.735	0.820	260	1540	7	7
Glucose	Y	7	0.735	0.730	230	1590	6.5	6.5
	Y+L.J	7	0.730	0.730	250	1610	7	7
	Y+L.R	7	0.730	0.780	280	1650	6.5	6.5
Fructose	Y	7	0.70	0.860	340	1654	7	6.8
	Y+L.J	7	0.708	0.890	380	1700	7.5	7.2
	Y+L.R	6	0.700	0.901	400	1740	7	6.8
Honey	Y	7	0.700	0.820	320	1620	8	7.9
	Y+L.J	6	0.740	0.847	350	1690	9	8.8
	Y+L.R	5	0.702	0.880	390	1720	8	7.6
Molasses	Y	8	0.750	0.910	370	1684	7.5	7.3
	Y+L.J	8	0.790	0.870	392	1700	8.5	8.3
	Y+L.R	7	0.790	1.19	420	1470	7.0	6.5
Dibis	Y	5.5	0.706	0.906	385	1710	8.5	8
	Y+L.J	5.5	0.711	0.870	400	1730	9	8.9
	Y+L.R	5.5	0.713	1.11	440	1800	8	7.5

(1): Yoghurt culture alone **(2): Yoghurt culture + *L. johnsonii***
(3): Yoghurt culture + *L. reuteri*.

Regarding the sensory evaluation, stirred yoghurt prepared with strain *L. johnsonii* as adjunct exhibited very good sensory properties and it had highest score. It had less acidic taste while the samples prepared with *L. reuteri* had traditional taste. Nerveless the yoghurt sweetened with sucrose or glucose had a weak structure as their viscosity is the least. Also the flavor and taste were enhanced when dibis and honey were added this was in agreement with Lászlóvarga (2006) who reported that honey decrease the sourness of solutions, this function can serve to acid product such as yoghurt.

Conclusions

The combination of probiotic cultures with standard yoghurt cultures can enhance sufficient acid production to develop the desired texture, flavor and aroma. Stirred yoghurt prepared with *L. johnsonii* had higher sensory score than the samples with or without *L. reuteri.* Enrichment yoghurt with dibis, molasse or honey is recommended, because they are natural sweeteners, they are preferable sweeteners for the two probiotic bacteria and yoghurt culture also they possess a wide range of beneficial nutritional properties. In addition they improved the sensory quality of the finished product.

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سلوك نمو بعض بكتيريا حمض اللاكتيك في اللبن الزبادي المقلب المضاف اليه بعض المحليات الطبيعية

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تم دراسة تأثير بعض المحليات مثل السكروز، الجلوكوز، فركتوز، العسل الأبيض، المولاس والدبس على نمو ونشاط كل من *L. bulgaricus, S. thermophilus, L. reueri and L. johnsonii* وذلك عن طريق تقدير كل من pH , OD أثناء فترة التحضين علي درجة الحرارة المثلي ولمدة ٢٤ساعة وذلك عند صفر، ٤، ٨، ٢٤ساعة وقد كانت أهم النتائج كالآتي:

أظهرت النتائج بصفة عامة أن *L. bulgaricus* كانت الأعلى في درجة النمو والنشاط يليها *S. thermophilus* أما بالنسبة لميكروبي *L. reueri, L. johnsonii* فقد كانا الأقل نموا ونشاطا في بيئة اللبن الفرز المضاف اليه المحليات الطبيعية المختلفة أما بالنسبة لتأثير المحليات المختلفة على نشاط الميكروبات المستخدمة فقد وجد ان الدبس والمولاس لهما تأثير مشجع لنمو *L. bulgaricus* أما الجلوكوز كان له تأثير مثبط لنشاط ميكروب *S. thermophilus* حيث كانت قيمة الـ pH ٤,٢٥ في نهاية مدة التحضين ولم تتأثر قيم الـ OD معنويا بنوع المحلى المستخدم وقد أظهرت النتائج أن المحليات المختلفة لم يكن لها أي تأثير تشجيعي أو تثبيطي على ميكروبي *L. reueri, L. johnsonii*

وجد ان هناك أنخفاض في أعداد جميع الميكروبات المستخدمه بمعدلات مختلفة و ذلك أثناء فترة التخزين تحت التبريد. و كانت الاعداد اعلي من ٥ دورات لوغارتمية خاصة في حالة اضافة الدبس.

في النهاية تم تصنيع زبادي مقلب ومضاف اليه المحليات سابقة الذكر باستخدام باديء الزبادي بالاضافة الى ميكروبي *L. reueri*, *L. johnsonii* , وتم تحليل الزبادي الناتج من الناحية الفيزوكيميائية والحسية وكانت أهم النتائج حصول الزبادي المصنع باستخدام ميكروب *L. johnsonii* على أعلى درجات التقييم الحسي وخاصة المضاف اليه الدبس أو المولاس أو العسل الأبيض.