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Properties of Non-Fat Yoghurt as Influenced by The Incubation Temperature of Exopolysaccharide Producing Culture

Seham Swelam^{1*}; M. A. Rashed¹; H. E. Hatem² and Eman F. khames²



² Animal Prod. Res. Institute, Agric. Res. Center, Egypt







Different incubation temperatures (40°C, 42°C and 45°C) were used in making non-fat yoghurt. Exopolysaccharides producing culture consists of Streptococcus thermophilus, Lactobacillus delbrueckii ssp. bulgaricus and Lactobacillus fermentum with (1:1) or without commercial yoghurt starter culture (YCx11) were used. Treatments were examined during the storage. Results showed that, the fermentation time decreased significantly by increasing the incubation temperature, further decrease of the incubation period was observed by using a combination of YC and EPS cultures. The higher pH value and lower acidity were observed by using EPS instead of YC cultures, while using higher incubation temperature improved development of the acidity. The highest acetaldehyde content and water holding capacity were achieved by using a mixture of the tested cultures at 42°C., followed by 45°C. The same trend was noticed for the viscosity parameter; as compared with the control (YC culture at 42°C), which had lowest value. Yoghurt made using EPS culture at 40°C resulted in a very high wheying-off, but it could be treated by the combination of EPS and YC cultures at the same temperature. More improvement, however, could be achieved by increasing the incubation temperature to 42°C. Panelists gave the highest sensory scores to the yoghurt made by a mixture of EPS and YC cultures at 42 and 45°C. The results revealed that the yoghurt made with EPS and YC starter cultures (1:1) at 42°C was the most accepted and were of the best rheological properties, compared with the other treated samples.

 $\textbf{Keywords:} \ Exopolysaccharides, Fermentation \ temperature, \ Yoghurt.$

INTRODUCTION

Exopolysaccharides (EPS) are high molecular-weight carbohydrates consist mainly from D-galactose, D-glucose and L-rhamnose. D-mannose can also be found in some EPS as well as in acetylated aminosugars, glucuronic acid, phosphate groups and acetyl groups. EPS are naturally produced by some lactic acid bacteria (LAB) during the fermentation process. Some bacteria produce only capsular EPS and some produce only slime (ropy) form whereas, in some cases, bacteria can produce both forms of EPSs (Yang, et al. 1999, Broadbent, et al. 2003). The EPS play an important role in the improvement of physical properties of fermented milks, which can be efficiently used as commercial stabilizers for preventing or reducing syneresis.

In addition, it provides the fermented milk products with suitable structure and viscosity. Usually, it acts like a stabilizer, viscosifier, emulsifier or gelling agent providing a product with natural thickness (Ruas-Madiedo and Reyes-Gavilan 2005). EPS produced by LAB cultures offer a natural way for making low- fat or fat-free fermented milks with more acceptable flavour and sensory attributes as well as an increased water-binding capacity. Furthermore, EPS of LAB are thought to have beneficial effects on human health such as cholesterol- lowering ability, anticanceral, immunomodulation and antitumoral activities and prebiotic effect (Dal Bello *et al.* 2001, Korakli *et al.* 2002 and Pigeon *et al.* 2002). The quantity of EPS depending on type of the lactic acid bacteria (LAB) and growing conditions (pH,

temperature and incubation time) (De Vuyst, *et al.*, 2001 and Broadbent *et al.*, 2003). The fermentation temperature and type of the used culture usually have a crucial role for the development of yoghurt quality and functional properties. Increased incubation temperature and EPS culture led to a higher water-holding capacity but lower syneresis, storage (G') and loss moduli (G"). Using EPS producing starter culture resulted in decreased syneresis, G' and G" and increased water holding capacity (WHC) of yoghurt gels compared with the non-EPS culture. (Abbasi *et al.* 2009). The fermentation temperature significantly contributes to EPS concentration because the increased rate of fermentation temperature was attributed to increased metabolic activity of LAB (Feldmane *et al.* 2014).

Chemical, rheological and sensory properties of non-fat yoghurt was studied as affected with incubation temperature.

MATERIALS AND METHODS

Cow's skim milk powder was taken from ValioLapinlahti Plant - Finland. It contains 1.25% fat, 52% lactose, 36% protein, 4% moisture and 8% ash.

Yoghurt starter culture (YC-x11) consisting of Streptococcus thermophilus and Lactobacillus delbrueckii ssp. bulgaricus was obtained from Chr. Hansen, Copenhagen, Denmark. Commercial exopolysaccharides producing cultures (EPS) Yo-Flex starter: Harmony 1.0 composed of Streptococcus thermophilus, Lactobacillus

* Corresponding author. E-mail address: sehamswelam9@gmail.com DOI: 10.21608/jfds.2019.71333 delbrueckii ssp. bulgaricus and Lactobacillus fermentum was obtained also from Chr.Hansen, Denmark.

Reconstituted skim milk powder (11%) was used in the manufacture of yoghurt which carried out using the method described by Tamime and Robinson (1999). The reconstituted heated milk in a water bath to 90°C/10 min. and divided into seven equal portions indicated as follows:

- Control: inoculated with 2 % yoghurt starter culture (YC) at 42°C.
- **Treatment** (1): inoculated with 2% EPS starter culture at 40°C.
- **Treatment (2):** inoculated with 1% EPS starter culture+ 1% YC at 40°C.
- **Treatment** (3): inoculated with 2% EPS starter culture at 42°C.
- **Treatment (4):** inoculated with 1% EPS starter culture+ 1% YC at 42°C.
- **Treatment (5):** inoculated with 2% EPS starter culture at 45°C.
- **Treatment (6):** inoculated with 1% EPS starter culture+ 1% YC at 45°C.

All of the samples were hold at the incubation temperature until complete coagulation, followed by cooling overnight at the refrigeration temperature 5±2°C. The activity of the used cultures was determined during the fermentation by measuring the development of the pH at the examined intervals until reaching the pH to about 4.6 (Swelam, 2018). The resultant yoghurt was chemically, rheologically and organoleptically evaluated when fresh and after 3 days, 7 days of storage period.

For determination of the titratable acidity, the method described by Ling, (1963) was used. The results were recorded as (%) of lactic acid.

The pH value was measured electrometrically using lab. pH meter (crison pH meter, Spain).

Acetaldehyde was detected as mentioned by Less and Jago (1969).

Viscosity (expressed as centipoise (cp)) was determined (after manually stirring of yoghurt gels) using a digital Brookfield viscometer (LVDV–E, Brookfield Eng. Lab., Middleboro, MA, USA) and spindle No. 63, at speed of 50 rpm. The rate of curd syneresis was measured using the drainage methods as described by Mehanna and Mehanna (1989).WHC of yoghurt was measured according to (Isanga and Zhang, 2009).

The sensory properties of yoghurt were assessed according to El-Shibiny, *et al.*,(1979).

Statistical analysis of the obtained results was carried out using in (SPSS, 1999).

RESULTS AND DISCUSSION

Results in Fig. (1) illustrate the insignificant differences in the pH values between all of the tested treatments at the beginning of the incubation time until 60 min. After 90 min until the end of fermentation period, the measured pHs varied significantly due to the incubation temperature. Using different cultures at the same temperature did not affect (P>0.05) the pH development. Thus, after 240 min of the fermentation time, the pH was 4.68 for control sample (2% yoghurt culture at 42° C), 4.85 for T3 (2% EPS at 42° C) and 4.63 for T4 (1% YC + 1% EPS at 42° C).

Regarding the impact of incubation temperature, it's clear to conclude that by increasing the incubation temperature, the fermentation time decreased which might be due to stimulate the EPS culture. Since after 240 min of incubation time, the measured pH of samples inoculated with 2% of EPS at 40, 42 and 45°C were 5.16, 4.85 and 4.81 respectively. Meanwhile, yoghurt fermented by 2% EPS starter culture at 40°C resulted in the longest fermentation time until pH~4.6 (240 min), while the lowest (210 min) was reported by T6 (EPS mixed by YC at 45°C). These results coincided with Feldmane et al., (2014) and Yilmaz, et al. (2015), who found fermentation temperature significantly contributes to EPS concentration because of the increased rate of fermentation temperature is attributed to the increased metabolic activity of lactic acid bacteria (LAB). And the lower pH values were achieved as the incubation temperature was increased from 32°C to 42°C.

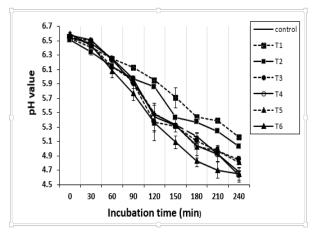


Fig. 1. pH profile during fermentation of yoghurt milk as affected by using exopolysaccharides producing culture (EPS) at different temperatures.

Fig. (2) reveals the effect of adding EPS at different temperatures on the pH of resultant yoghurt. Significant differences between the pH of yoghurt samples related to the used culture and incubation temperature. Yoghurt samples made from 2% EPS and incubated at 40°C had higher (P≤0.05) pH value compared to the samples made with 2% of EPS culture being incubated at 42 and 45°C. The corresponding recorded values were 5.14, 4.86 and 4.73, respectively.

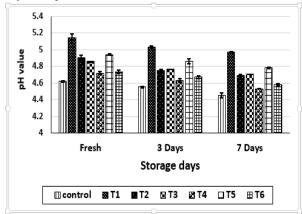


Fig .2. pH of non-fat yoghurt as affected by using exopolysaccharides producing culture (EPS) at different temperatures.

It's clear from the obtained results that the use of EPS instead of yoghurt starter culture increased ($P \le 0.05$) the pH of the final product. The pH was 4.62 for the control sample, while it was 4.86 in case of using EPS culture with the same level (2%) and the same incubation temperature (42°C). The pH significantly decreased when the EPS were combined with yoghurt culture by 1:1 (4.72), compared by using 2% of EPS at the same incubation temperature (42°C). The pH values gradually decreased by the progress of the storage period. Similar results are obtained by GüLER-AKIN, *et al.*, (2009) and Feldmane, *et al.*, (2014).

Data presented in Fig. (3) show the change in the titratable acidity of non-fat yoghurt made with EPS producing starter cultures and yoghurt starter culture at different temperatures during storage period for 7days. Regarding the impact of using EPS, it could be noticed that by using EPS culture (2%) instead of YC at 42°C the acidity decreased from 0.74% (control sample) to 0.66%. The acidity increased significantly to reach 0.76% in case of using a combination of yoghurt starter culture with EPS (1:1). On the other hand, an increase of the incubation temperature accompanied with an increase of the acidity either when EPS was used alone or mixed with yoghurt starter culture. A gradual increase in acidity was detected by advancing the storage period in all treated samples (Salama 2002, Badran, et al., 2004 and Chramostová et al., 2014).

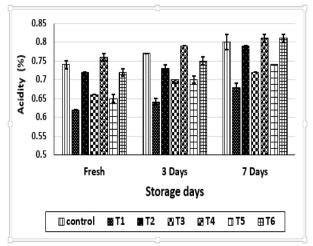


Fig. 3. Acidity of non-fat yoghurt as affected by using exopolysaccharides producing culture (EPS) at different temperatures.

Acetaldehyde contents of the resultant yoghurt are presented in Fig (4). It is obvious that, acetaldehyde content was affected significantly by using the tested cultures and incubation temperatures. The highest acetaldehyde content was observed by using a mixture of EPS and yoghurt starter culture (1:1) at 42°C (14.00) µmole/100g, followed by the mixture cultures at 45°C (12.87) µmole/100g as compared with the control sample (12.61) µmole/100g. Whereas, the lowest content of acetaldehyde was recorded by T1 (2% EPS at 40°C), followed by T5 (2% EPS at 45°C). In contrast, ropy or viscous strains produced low levels of acetaldehyde. From the previous results, it could be concluded that using the mixture of cultures improved the production of acetaldehyde and further production was achieved by increasing the incubation temperature.

Ott *et al.* (2000) and Bongers *et al.* (2004) reported that non EPS-producing strains of yoghurt bacteria produced

high levels of acetaldehyde. The storage progress affected negatively the acetaldehyde content since the lowest acetaldehyde content was noticed at the end of the storage period (7 days) for all samples. The decrease of acetaldehyde during storage might probably be due to its conversion into another organic compounds such as ethanol or diacetyl (Elloly and El- Hofi, 1999).

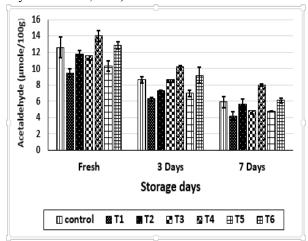


Fig. 4. Acetaldehyde content (µmole/100g) of non-fat yoghurt as affected by using exopolysaccharides producing culture (EPS) at different temperatures.

Concerning viscosity (Fig 5), it could be seen that raising the incubation temperature and using EPS producing culture resulted in an enhancement of the viscosity of the resultant yoghurt. The highest mean of the viscosity was determined in the samples inoculated with a mixture of the used cultures (1:1) at 42°C (5131 Cp), followed by 45°C (5123 Cp). The control sample had the lowest (P≤0.05) viscosity value of 3682Cp. The viscosity of all samples increased continuously throughout the storage period. The obtained results came in harmony with those mentioned by Sebastiani and Zelger (1998), Marshall and Rawson (1999), and Hassan, *et al.*, (2002).

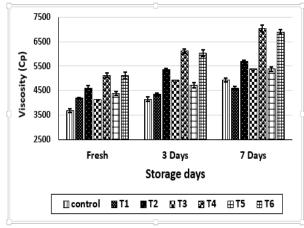


Fig. 5. Viscosity (Cp) of non-fat yoghurt as affected by using exopolysaccharides producing culture (EPS) at different temperatures.

Results of the water holding capacity (WHC) are shown in Fig (6). The WHC value was found to be higher in yoghurt made using 2% EPS and incubated at temperature at 42°C. (48.32), compared to (46.92) at 45°C and (46.51) at 40°C. As a consequence of combination of

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EPS and yoghurt starter culture, WHC increased to reach the maximum when the samples were incubated at 42°C (52.21), followed by (51.06) at 45°C.The lowest values were determined for control, T1 (2% EPS at 40°C) and T5 (2% EPS at 45 °C) with insignificant differences among them .WHC increased significantly by increasing the storage period for all treated samples. The present results agreed with Abbasi *et al.*, (2009), who stated the EPS treatments had higher WHC than non-EPS treatments, which might be explained by the higher WHC of EPS.

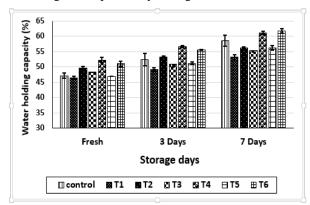


Fig. 6.Water holding capacity (%) of non-fat yoghurt as affected by using exopolysaccharides producing culture (EPS) at different temperatures.

Changes in curd syneresis (CS) of yoghurt samples as affected the applied treatments are indicated in Fig. (7).

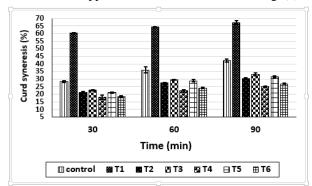


Fig. 7. Curd syneresis (%) of fresh non-fat yoghurt as affected by using exopolysaccharides producing culture (EPS) at different temperatures.

Fresh yoghurt made with EPS producing starter cultures at 40°C resulted in a great wheying off (60.34), followed by the control one (28.42%). The curd syneresis

decreased significantly to reach 21.07% in case of using a combination of yoghurt starter culture with EPS (1:1) at the same incubation temperature.

The lowest (p≤0.05) wheying off was noticed by using a mixture of cultures (1:1) and with increase of the incubation temperature to 42°C (T4). From the previous data it could be concluded that yogurts made by adding EPS starter cultures at 42 and 45°C resulted in lower level of syneresis, compared to yogurts made using EPS at 40°C.

Similar trend was recorded in case of the yoghurt after 7 days of storage. However, syneresis decreased for all yoghurt samples after 7 days of storage period. This could be as a result of metabolic activity of yoghurt starter cultures, and the decrease in net pressure in the protein matrix, which decreases the syneresis (De Vuyst and Degeest, (1999) and (Güler-Akın, et al., 2009). However, EPS have the ability to bind water which counteracts the negative effect of the open structure. yoghurts made using EPS culture had a lower level of syneresis than those produced with non-EPS culture at temperatures 37, 42 and 45°C. According to Abbasi, et al., (2009), Cerning, (1990), EPS producing cultures resulted in higher WHC and lower level of syneresis than those produced with non-EPS culture at temperatures 37, 42 and 45°C, furthermore exopolysaccharide culture and decreased incubation temperature decreased the gel syneresis.

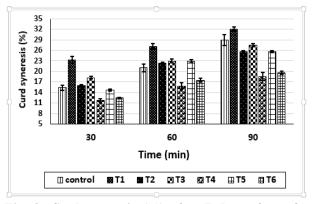


Fig. 8. Curd syneresis (%) after 7 days of non-fat yoghurt as affected by using exopolysaccharides producing culture (EPS) at different temperatures.

The sensory properties of non-fat fresh yoghurt as affected by adding EPS producing culture at different temperatures are presented in Table (1).

Table 1. Sensory properties of fresh non-fat yoghurt as affected by using exopolysaccharides producing culture (EPS) at different temperatures.

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Properties	Control	T1	T2	Т3	T4	T5	T6	
Appearance (10)	8.67±0.33 ^A	5.33±0.33 ^B	6.00 ± 0.58^{B}	8.67±0.33 ^A	9.00±0.00 ^A	8.67±0.33 ^A	9.00±0.58 ^A	
Firmness (10)	8.67 ± 0.33^{AB}	3.67 ± 0.88^{C}	7.33 ± 0.67^{B}	8.00 ± 0.00^{AB}	9.67 ± 0.33^{A}	8.00 ± 0.58^{AB}	9.33 ± 0.33^{A}	
Smoothness (10)	8.00 ± 0.00^{B}	6.00 ± 0.58^{C}	8.00 ± 0.58^{B}	9.33 ± 0.33^{A}	7.67 ± 0.33^{B}	9.33 ± 0.33^{A}	7.67 ± 0.33^{B}	
Wheying off (10)	8.33 ± 0.33^{AB}	5.67 ± 0.33^{C}	8.00 ± 0.58^{B}	9.00 ± 0.58^{AB}	9.67±0.33 ^A	8.67 ± 0.33^{AB}	9.50 ± 0.29^{A}	
Flavour (60) Acid (10) Bittemess (10) Flat (10) Foreign (10) Cooked (10) Unclean (10)	9.67±0.33 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A	8.33±0.33 ^B 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A	9.00±0.00 ^{AB} 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A	8.33±0.33 ^B 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A	9.67±0.33 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A	8.50±0.29 ^B 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A	9.33±0.33 ^{AB} 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A	

^{*}Averages with different superscripts (A,B,C...etc.) differed significantly (p \leq 0.05). *control:(2 % starter culture at 42°C) , T1: (2% EPS starter culture at 40°C), T2:(1% EPS starter culture+ 1% starter culture at 40°C), T3:(2% EPS starter culture at 42°C), T4:(1% EPS starter culture+ 1% starter culture at 42°C), T5: (2% EPS starter culture at 45°C), T6:(1% EPS starter culture+ 1% starter culture at 45°C).

It is obvious that all sensory characters were the lowest (P≤0.05) by applying T1 (EPS at 40°C), followed by T2 (EPS with YC at 40°C). It could also be noticed that there was no significant difference for general appearance, firmness, smoothness and wheying-off properties among control sample and the treated samples by either EPS single culture or mixed with yoghurt starter culture at 42 or 45°C. Since, it is clear from pre-mentioned data that using a lower incubation temperature (40°C) with EPS suffer from very weak structure (firmness, 3.67) and a huge wheying off since they were ranked the lowest scores in this respect (5.67).For flavour properties, the lowest scores for acid flavour were recorded by T1 (EPS at 40°C) and T3

(EPS at 42°C) whereas the highest values were reported for control sample and T4 (EPS mixed with YC at 42°C). All samples were free of off-flavour.

The same trend of the presented results was observed after 7 days of storage for all treated samples Table (2). In addition, all measured properties ranked higher scores, compared to those given by the fresh samples expect of acidity, which it was almost bite lower.

The firmness of fermented skim milk made using capsular EPS-producing or ropy EPS-producing cultures was lower than that of fermented skim milk made with non- EPS-producing starter cultures (Hassan, *et al.*, 2003) and Amatayakul *et al.* (2006).

Table 2. Sensory properties of 7 day non-fat yoghurt as affected by using exopolysaccharides producing culture (EPS) at different temperatures.

properties	Control	T1	T2	Т3	T4	T5	T6
Appearance (10)	9.33±0.33 ^A	5.33±0.33 ^B	8.33±0.33 ^A	9.33±0.33 ^A	9.67±0.33 ^A	9.33±0.67 ^A	9.67±0.33 ^A
Firmness (10)	8.67 ± 0.33^{AB}	5.67 ± 0.33^{C}	8.00 ± 0.58^{B}	8.67 ± 0.33^{AB}	9.67 ± 0.33^{A}	8.67 ± 0.33^{AB}	9.67 ± 0.33^{A}
Smoothness (10)	8.67 ± 0.33^{AB}	6.00 ± 0.00^{C}	8.00 ± 0.58^{AB}	9.00 ± 0.33^{A}	7.67 ± 0.33^{B}	9.00 ± 0.58^{A}	7.67 ± 0.33^{B}
Wheying off (10)	8.67 ± 0.67^{AB}	6.00 ± 0.58^{C}	7.67 ± 0.33^{B}	9.00 ± 0.58^{AB}	10.00±0.00 ^A	9.00 ± 0.58^{AB}	9.67 ± 0.33^{A}
Flavour (60) Acid (10) Bitterness (10) Flat (10)	8.67±0.33 ^{AB} 10.00±0.00 ^A 10.00±0.00 ^A	7.67±0.33 ^B 10.00±0.00 ^A 10.00±0.00 ^A	8.50±0.29 ^B 10.00±0.00 ^A 10.00±0.00 ^A	8.33±0.33 ^B 10.00±0.00 ^A 10.00±0.00 ^A	9.67±0.33 ^A 10.00±0.00 ^A 10.00±0.00 ^A	8.33±0.33 ^B 10.00±0.00 ^A 10.00±0.00 ^A	9.67±0.33 ^A 10.00±0.00 ^A 10.00±0.00 ^A
Foreign (10) Cooked (10) Unclean (10)	10.00±0.00 ^A 10.00±0.00 ^A 10.00±0.00 ^A						

^{*}see legend to Table (1) for details

CONCLUSION

It could be concluded from this study that, the change in incubation temperature of EPS-producing culture significantly affected the properties and quality of non-fat yoghurt. The best viscosity, water holding capacity and sensory attributes with the lowest whey-off can be achieved by using a combination of EPS and YC starter culture (1:1) at 42°C followed by 45°C. Whereas, the used of EPS alone at lower temperature (40°C) defected (P≤0.05) the properties of the resultant yoghurt.

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تأثير درجة حرارة التحضين للبادئ المنتج للسكريات العديدة علي خواص اليوجورت الخالي من الدهن سهام سويلم أ ، مصطفى على راشد أ ، حامد السيد حاتم 2 و ايمان فؤاد خميس 2 اكليه الزراعة جامعة كفر الشيخ قسم علوم الالبان عمركز البحوث الزراعية معهد بحوث الانتاج الحيواني

تم اجراء هذا البحث لدراسة تأثير درجات الحرارةالمختلفة (40 ،22م وكم") في صناعة اليوغورت الخالي من الدهن و تمت عملية التخمر باستخدام البلائ المنتج للسكريات العديدة والذي يتكون من سلالات Streptococcus thermophilus ويقاس درجة نشاط البلائ عن طريق تتبع التغير في الرقم الهيدروجيني اثناء fermentum وتم استخدامه منفودا او مخلوطا مع بلائ اليوغورت التقليدي بنسبة (1:1) تم قياس درجة نشاط البلائ عن طريق تتبع التغير في الرقم الهيدروجيني اثناء التخمر وجيني اثناء التخمر في المعتمدين اليوغورت النتج كيماويا ، ريولوجيا وحسيا وذلك عندما كان طازجا وبعد 3 و7 ايام من البلائ المنتج للسكريات العديدة وبلائ اليوغورت التقليدي بينما أدي استخدام درجة الحرارة الأخفاض معنويا عن طريق استخدام خليط من البلائ المنتج للسكريات العديدة وبلائ اليوغورت التقليدي بينما أدي استخدام درجة الحرارة الأعلي في التحصين الي التحسين من انتاج الحامض كما تم تحقيق اعلي محتوي من الاسبتالدهيد ومقدرة مسك الخثرة الماء باستخدام خليط من السلالات المستخدمة وذلك علي درجة حرارة 40 م ولوحظ نفس الاتجاه للزوجة وقد حصل الكنترول (المصنع باستخدام بلدئ اليوغورت التقليدي علي 42° م ولوحظ نفس الاتجاه للزوجة وقد حصل الكنترول (المصنع باستخدام بلدئ اليوغورت المرد الشرش ولكن أمكن معالجة ذلك بوضوح أن اليوغورت المحريات العديدة وبلائ اليوغورت النقليدي علي درجة حرارة 40° م كان يعاني من اعلي جودة لليوغورت بزيادة درجة حرارة اللام باستخدام خليط من الدي العديدة وبلدئ اليوغورت التقليدي بنسبة المرد والموضع من بدي المدي والدئ اليوغورت التقليدي بنسبة العديدة والدوغورت التقليدي على درجة حرارة 42° م كان يعاني من المع باستخدام خليط من البلائ اليوغورت التقليدي بنسبة العديدة وبلائ اليوغورت المعاملات .