Quality evaluation of bifidus yoghourt and its microbial activity

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Abstract

This study was planned to evaluate and compare the quality of traditional yoghurt (Y) which was made with traditional yoghurt cultures (3%), Bifidus yoghurt (Y_1) made with 1.5% traditional yoghurt cultures + 1.5% B. longum.The samples were prepared and analyzed for physico-chemical and organoleptic properties. Physico-chemical analysis reveled that coagulation time in (Y) was shorter than (Y_1) (3 hr:30 min) and (4 hr: 45 min) respectively. Acidity in (Y) sample had the highest acidity 1.12 and 1.42 after 7 and 14 days respectively while (Y_1) samples had lower acidity 0.92 and 1.08, after 7 and 14 day respectively. Even after 21 days, acidity was still not high 1.2 in (Y_1) samples .The overall acceptability of organoleptic examination of prepared yoghurt during storage period recorded that (Y) sample had the highest score points 9 and decreased to be 7.5 and 6 after 7 and 14 days of storage, respectively, while the scores of (Y_1) samples slightly increased by cold storage to be 9 on the 1st day then decreased gradually but became higher than that of (Y) samples as, they gained scores of 8 and 7.5 after 14 and 21 days, respectively and maintained there high scores till the end of, storage period. The effect of addition of Fractooligosaccharid 1.5% to bifidus yoghurt (Y_2) on the numbers of *Bifidobacteria longum* yoghurt samples, showed rapid increase during coagulation and storage period, from 8.4 and 8.2 $\log_{10}$ cfu/g to be 9.54 and 9.5 $\log_{10}$ cfu/g in (Y_1) and (Y_2), respectively.

In determination of the effectiveness of traditional yoghurt (Y) and bifidus yoghurt(Y_1) and with addition of FOS (Y_2) on inactivation of E. coil (NCTC 2923 - ATCC 8739) in processed samples, the results revealed that (Y) and (Y_1) yoghurt samples had the highest inhibitory effect than (Y) against *E. coli*. However, the combination of traditional yoghurt culture and *bifidobacterium longum* with addition of prebiotic (FOS) could be used as an effective method to increase the quality and improve the biosafety of the product.

Key words: *Bifidobacteria longum , Bifidus yoghurt, Fractooligosaccharid, prebiotic (FOS).*

Introduction

Fermented milk are considered as one of the oldest dairy products consumed widely all over the world and the fermentation is initiated by the growth of lactic acid bacteria for the production of lactic acid from lactose. Yoghourt is the most popular fermented milk product and its taste and aroma vary from brand to another.

Probiotics have been defined by the Food Agricultural Organization / World Health confer a health benefit to host" **(Brown and Valiere, 2004).**

Probiotics are nonpathogenic microorganisms that, when ingested, exert a positive influence on the health or physiology of the host. They can influence intestinal physiology either directly or indirectly through modulation of the endogenous ecosystem or immune system. The results that have been shown with a sufficient level of proof to enable Probiotics to be used as treatment for gastrointestinal disturbances as 1) to prevent or shorten the duration of antibiotic – associated diarrhea, 2) to prevent further recurrence of infection associated diarrhea, and 3) to shorten the duration of diarrhea in infants with rotavirus enteritis and probably also in gastroenteritis of other causes (**Marteau *et al.*, 2001).**

Bifidobacteria were grouped into 24 spp. But only five spp. of them *(B. bifidum , B. lognum , B. infantis , B. breve and B. adolesentis)* have attracted attention in the dairy industry for the

manufacture of functional fermented milk products **(Tamime *et al*., 1995)** .

Bifidobacterium longum characterized by a high intestinal colonization and acid resistance characters which make them useful for incorporation in fermented dairy products **(Kheadr *et al.,* 2002)**.

The importance of *Fructooligosccharide (FOS)* as prebiotic substance based not only on its enhancing of *Bifidobacteria*l growth but also, it has several beneficial effects on human health as enhancing the absorption of minerals and reduction of constipation, serum cholesterol level, obesity and cancer risk **(Roberfroid, 2000 , 2001).**

Bifidobacteria and different types of *Lactobacilli* showed highly antimicrobial activity against *E.coli* and *S.typhi* **(Kim *et al.,* 2002)**. *Bifidobacterial* of human origin inhibit the growth of *E.coli* and reduce its adhesion to gastrointestinal tract as well as their resistance to acidic, bile and hydrogen peroxide **(Gagnon *et al.*, 2004).**

Makras and De Vuyst (2006) recorded that *Bifidobacterial* had strong inhibitory activity towards Gram negative bacteria mainly, *E.coli* and *Salmonella spp*. due to the lowering of the pH and the production of organic acids mainly acetic and lactic acids.

This work was carried out to study the following:-

1-Evaluation of the difference between the traditional yoghurt and bifidus yoghurt quality and shelf life

2-The effect of *FOS* "on the growth of *B. longum* in bifidus yoghurt .

3-Fate of *E.coli* in the bifidus yoghurt with or without addition *FOS*.

Materials and methods

I- Preparation of yoghurt samples

1-Yoghurt cultures:

- FD-DVS,YC-X11 strain culture contain *Streptococcus thermophiles & Lactobacillus delbrueckii subspp. Bulgaricus* (1:1) was obtain from Agricultural Research Center, Giza, Egypt.
- ATTCC15707 strain culture contain *Bifidobacterium longum* was obtain from MIRCEN Faculty of Agricultural Ain-Shams Univ. Yoghurt cultures were prepared according to **Hull and Robert (1984).**

2-*E. coli* NCTC 2923 - ATCC 8739 was obtain from Food hygiene department -

Animal health Research Institute was prepared: according to **Zhao *et al*., (2004).**

3-Preparation of traditional yoghurt: according to **Nighswonger *et al*., (1996).**

4-Bfidus yoghurt preparation: according to **Nighswonger *et al*., (1996).**

5-Yoghurt preparation with addition fructooligosccharide (FOS): according to **Aryan and Poula (2007).**

II- Physico-chemical and Organoleptic Examination.

One sample was taken from each sample at zero time (from fresh samples) then after 1, 3, 5, 7, 14 and 21 days of cold storage.

1- Determination of coagulation time according to **(Ebing and Rutgers, 2006).**

2-Organoleptic examination**:** according to **Anna (1998)** by using the 9-point hedonic scale.

3-Determination of titratable acidity: according to **(Michael and Joseph, 2004).**

III- Microbiligical examination:

One sample was taken from each sample at zero time (from fresh samples) then after 1, 3, 5, 7, 14 and 21 days of cold storage.

1-Determination of Bifidobacterium longum count: according to **Dave and Shah, (1996).**

2-*E.coli* count on TBX: according to **ISO 16649-2: (2001).**

Results and Discussion

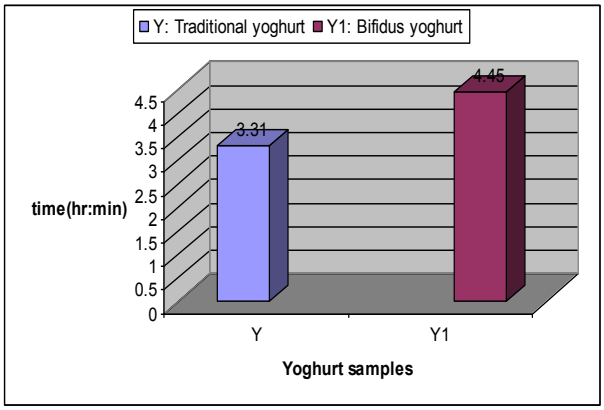

Fig (1). The mean values of coagulation time of yoghurt samples

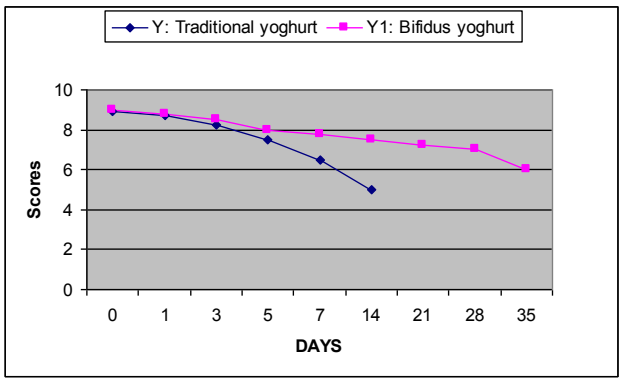

Fig. (2). Appearance mean scores of yoghurt samples.

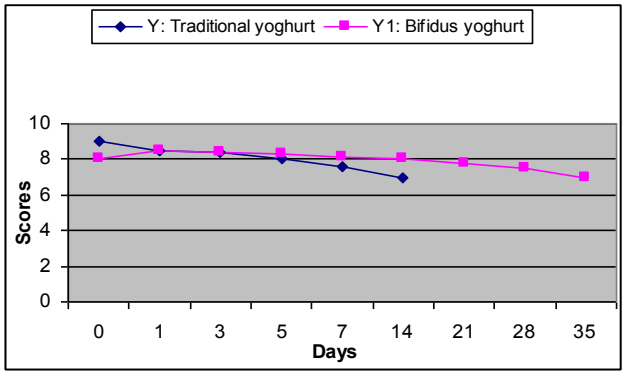

Fig. (3). Body and texture mean scores of yoghurt samples

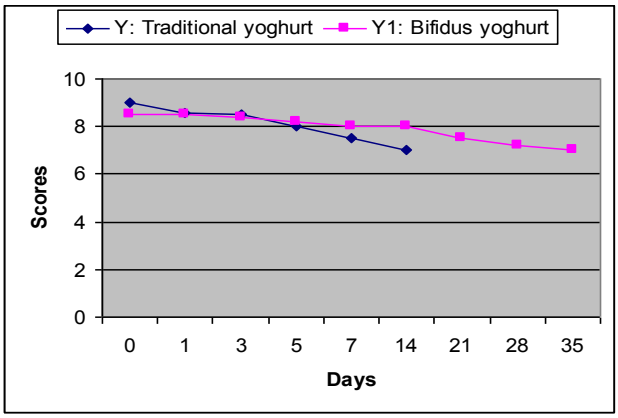

Fig. (4). Flavour mean scores of yoghurt samples

Table (1). The mean values of titratable acidity in the prepared yoghurt samples during refrigerated storage (mean±SE).

Samples	Traditional yoghurt	Bifidus yoghurt
Fresh	0.84±0.11	0.80±0.006
1	0.86±0.012	0.82±0.006
3	0.89±0.012	0.85±0.003
5	0.92±0.006	0.588±0.006
7	1.12±0.052	0.92±0.067
14	1.42±0.055	1.08±0.030
21	--	1.20±0.033

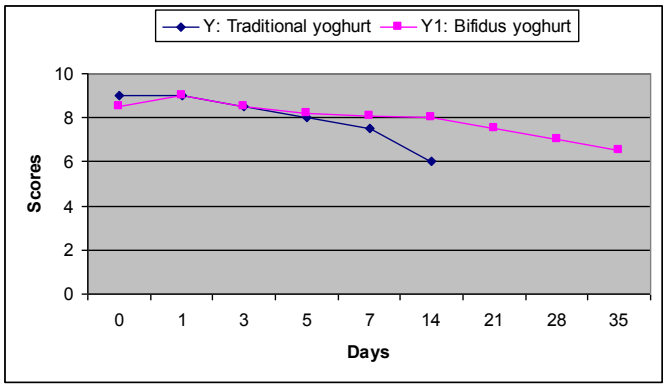

Fig. (5). Over all acceptability organoleptic mean values of yoghurt samples

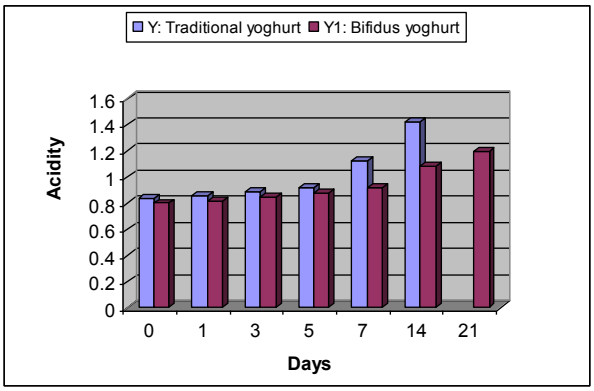

Fig (6). The mean values of titratable acidity

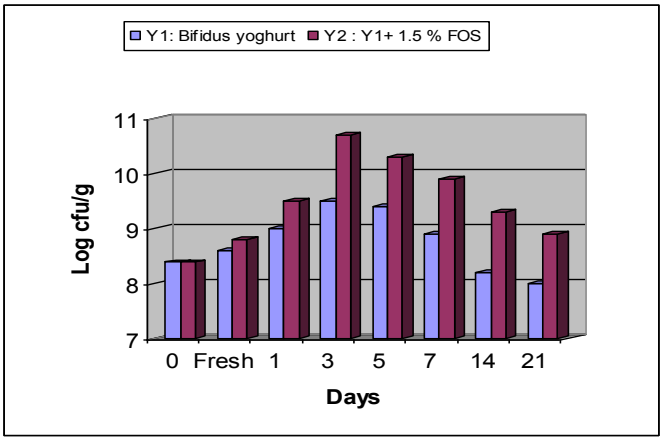

Fig. (7). Effect of FOS on the viability *B.longum* in yoghurt samples.

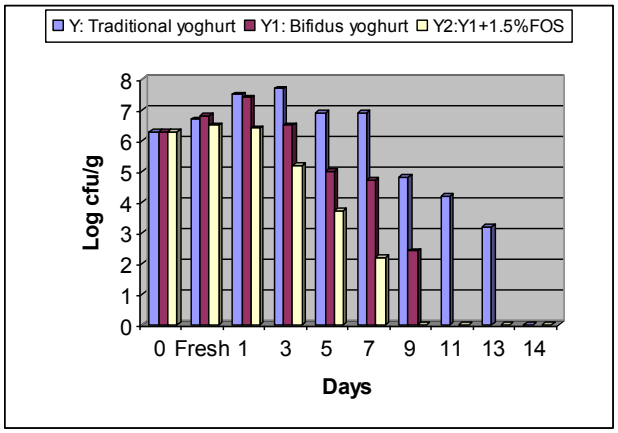

Fig (8). Viability of *E.coli* in yoghurt samples.

I-Bifidus yoghurt quality and shelf life

1- Coagulation time of prepared yoghurt samples:

Coagulation of milk occurs as a result of the precipitation of milk casein in the acidic condition at pH around 4.6 **(Lee and Lucey, 2004, 2010).**

Fig (1) showed that the Coagulation time varied widely according to starter cultures used: traditional yoghurt (Y) made with 3% yoghurt cultures (*Streptococcus thermophiles & Lactobacillus bulgaricus*) showed faster acid production and consequently shorter incubation time compared to bifidus yoghurt (Y_1) made with traditional yoghurt cultures 1.5 % + 1.5% *Bifidobacterium longum* .The increasing of Coagulation time in (Y_1) than (Y) may be due to used lower amount of traditional yoghurt cultures 1.5 % compared to that in (Y) sample 3% and the slow rate of Bifido bacterial growth with low acid production **(Shihata and Shah , 2000)**.

2- Organoleptic examination of prepared yoghurt

Fig (5) showed that the prepared fresh samples (Y) gave the highest mean score than (Y_1).

All samples showed gradual decrease in their scores, while on the 5th day of storage (Y) sample has lower scores than (Y1), After 14 day (Y) sample gained minimum score. Generally scores of sensory evaluation decreased during storage time, this could be attributed to the development of acidity and proteolytic activity of starter culture bacteria **(Aly *et al.*, 2004; Hussain *et al.*, 2009)**.

3- Titratable acidity of yoghurt sample.

Acidification of milk is primarily depend on conversion of Lactose into organic acids which

lowers the pH of milk from a value of 6.8 or less to less than 4.6 , thus protecting the fermented milk against The risk of contamination by different pathogens and making it hygienically safe **(Garbutt, 1997** and **AL–Kadamany *et al*., 2002).**

Data presented in Table (1) & Fig (6) illustrated the changes of acidity during the refrigerated storage. The acidity of all prepared yoghurt sample showed significant increase during the storage time. (Y) samples showed the highest titratable acidity value.

The mean value of titratable acidity of all samples increased during the storage period but (Y) samples showed higher values than (Y_1). The higher acidity in (Y) samples than those (Y_1) samples my be due to the (Y) samples contain amount of initial inoculums 3% in (Y) compared to 1.5% in (Y_1) samples in addition to the slow rate of Bfidobacteria in production of acidity **(Dave and Shah, 1997d)**.

The development of acidity was more progressively during the coagulation time if compared with that during the storages time. This give harmful effect on the viability of Bifidobacterial starter cultures **(Donkor *et al.*, 2007).** Therefore to ensure the activity of the culture and the PH must be controlled by buffering the yoghurt or terminating the inoculation period at PH 4.9 -5.0,

II- Microbiological examination

Effect of FOS on the growth of *B. longum.*

As shown in fig (6) the mean count of *B. longum* was 8.3 and 8.1 $\log_{10}$ cfu/g at zero time and increased to 8.6 and 8.8 $\log_{10}$ cfu/g in fresh (Y1) and (Y2) samples respectively .

After 3 days of storage*; B. longum* reached to maximum levels with mean count of 10.7 and 9.5 $\log_{10}$ cfu/g in (Y_2) and (Y1) samples respectively. Count of *B. longum* decreased gradually to be 8.9, 8.1 and 7.9 $\log_{10}$ cfu/g in (Y_1) sample and to 9.9, 9.3 and 8.9 $\log_{10}$ cfu/g in (Y_2) sample after 7, 14 and 21 days of storage, respectively.

From these results it was noticed that the growth of *Bfidobacteria* were increased by addition of FOS substance. These results agreed with **(Bouhnik *et al.,* 2004, 2006 and 2007)** which Concluded that the bifidogenic nature of FOS played an important role in increasing the growth of *Bifidobacteria.*

III – Fate of *E.coli* bacteria in Bifidus yoghurt without FOS.

In results given in fig (7), it was found that the initial count of E. coli increased during the coagulation time in sample (Y&Y_1) from 6.3 $\log_{10}$ cfu/g to be 6.9 and 6.8$\log_{10}$ cfu/g respectively and increased at the first day of storage to 7.7 and 7.5 $\log_{10}$ cfu/g respectively.

The count of *E.coli* decreased gradually in (Y_1) samples after the 3rd day of storage with mean values of 6.5 $\log_{10}$ cfu/g and decreased to 4.7 $\log_{10}$ cfu/g respectively after 7 day of storage and disappeared completely on the 11th day of storage.

While in (Y) sample; The count of *E.coli* still high to the 3rd day of storage with mean value of 7.7 $\log_{10}$ cfu/g and began to decrease to 6.9 , 6.9 , 4.2 and 3.2 $\log_{10}$ cfu/g after 5 , 7, 9 , 11 and 13 days of storage, respectively and not detected in the 14th day.

From obtained results we can concluded that the number of inoculated *E.coli* decreased by the end of storage period and the best reduction effect was observed in (Y_1) samples than (Y) samples. Similar results were reported by (**Farghaly, 2004** and **Amer *et al*., 2010).**

It seems that the presence of E.coli and its survival at both low temperature and pH confirmed the implication of acidic food on some outbreaks due to *E.coli* infection **(Sharp *et al*., 1995)**.

The effect of FOS on the viability of *E.coli* in bifidus yoghurt made with *B. longum* was illustrated in fig (7) showed that the initial count of E.coli was 6.5 $\log_{10}$ cfu/g then increased during the coagulation time to 6.5 $\log_{10}$ cfu/g. The count of E.coli in (Y_2) sample began to decrease from the 1st day of storage with mean count of 6.4 $\log_{10}$ cfu/g and the reduction in its count continued to be 5.2, 3.8 and 2.2 $\log_{10}$ cfu/g after 3, 5, and 7 day of storage, respectively and disappeared on the 9th day of storage .

It was noticed that the addition of FOS in (Y_1) samples inhabit *E.coli*, more than samples

without FOS.

Kapiki *et al.*, (2007) recorded that an infant formula containing a small quantity of prebiotic fractooligosaccharides (0.49/ d1) accepted and lead to rapid growth of Bifidobacteria in the gut of infants while decreased the number of pathogenic microorganism as *E.coli*.

Conculusion

Bifidus yoghurt samples recorded higher degrees for organoleptic evaluation and a higher bactericidal effect against *E.coli* than the traditional yoghurt. Supplementation of bifidus yoghurt with FOS increased the viability of *B.longum* and increased their antimicrobial effects.

Recommendation

Using of *B.longum* with yoghurt cultures in the manufactures yoghurt and supplementation of yoghurt with FOS improve the quality and safety of the yoghurt.

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تقييم جودة زبادى البيفيدو ونشاطه الميكروبى

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الملخص العربى
تم تصنيع عينات من الزبادي العينة الاولي Y تم تصنيعها من 3 % من بكتريا بادي الزبادي فقط والعينة الثانية (Y_1) تم تصنيعة من بادي الزبادي (1.5%) + بكتريا يفيدو لونجم تم تحضين المنتجات في درجة حرارة (40م – 42م) و حفظها في الثلاجة وقد تم اخذ عينات من اللبن قبل التحضين مباشرة ثم بعد التحضين وتكون الخثرة واثناء فترة الحفظ وكانت النتائج كالاتي :- تم تحديد فترة التحضين لكل منتج من المنتجات وقد لوحظ ان فترة التخضين للزبادي المصنع من بادي الزبادي فقط كانت 3 ساعات ونصف وا المجوعة (Y_1) 4 ساعات و45 دقيقة.
وقد تم فحص المنتجات من حيث الشكل الظاهري والطعم والرائحة بعد فترة التحضين وكذلك أثناء فترة الحفظ. ولقد وجد ان فترة تحضين الزبادي المصنع من بادي الزبادي فقط (Y) قد اعطي درجات تحكمية 9 اعلى من المنتج (Y_1) ثم بدات تتناقص اثناء فترة لتسجل 5 .7 و 6 بعد14 يوم من الحفظ في المنتجات (Y) ,(Y_1)علي التوالي وبعد 21 يوم كانت نتائج الفحص الظاهري 7.5 في المنتج (Y_1) علي التوالي اما المنتج (Y)فقد ظهرت علية علامات الفساد ينمو الفطريات علي السطح .
تم قياس الحموضة لكل المنتجات بعد انتهاء فترة التحضين مباشرة وتكون الخثرة واثناء مدة الحفظ وقد لوحظ ان الحموضة في المنتج (Y) حيث سجلت 1.12و 1.42 بعد 7 و 14 يوم علي التوالي اما في المنتج (Y_1)كانت الحموضة 0.89 و 0.92 كما ان عدد بكتريا يفيدو لونجم قد ازداد اثناء فترة التحضين ولكن الزيادة اصبحت ملحوظة ومستمرة اثناء فترة الحفظ ليصل الي اعلي مستوي له في اليوم الثالث حيث سجلت 9.6 في المنتج (Y_1) وقد لوحظ ان بكتريا يفيدو لونجم قد استمرت الزيادة فى مستوي العددي حتي اليوم السابع .
تم تصنيع منتجات الزبادي (Y_1)مع اضافة مادة فركتوأوليجوسكريد بنسبة 1.5% (Y_2)وتم العد البكتيري للبايفيدوبكتيريا وقد اظهرت النتائج ان عدد بكتيريا البايفيدو اعلي في العينات التي تحتوي علي مادة فركتوأوليجوسكريد (Y_2) مقارنة بالمجموعات الخالية من مادة فركتوأوليجوسكريد .
تم تصنيع منتجات الزبادي Y و (Y_1)مع اضافة عدد معلوم من بكتريا الايشريشيا كولاى وقد اوضحت النتائج ان ميكروب الايشريشيا كولاى له القدرة علي المقاومه في عينات الزبادي المصنعة من بادي الزبادي والعينات المصنعة من البايفيدو بكتريا علي السواء فقد زاد العدد اثناء فترة التحضين واثناء فترة الحفظ من 6,3 الى 7,65و7,47 على التوالى.ثم بدا العد يتناقص تدريجيا حتى تم القضاء علية تماما بعد11 يوم فى المنتج (Y_1)وفى اليوم14 للمنتج(Y).
تم تصنيع منتجات الزبادي السابقة مع اضافة عدد معلوم بكتريا الايشريشيا كولاى مع اضافة مادة فركتووليجوسكريد بنسبة 1.5%. فاظهرت نتائج العينات ان عدد ميكروب الايشريشيا كولاى قد ازداد في جميع العينات اثناء فترة التحضين والحفظ ولكن الزيادة كانت ملحوظة في العينات بدون فركتووليجوسكريد و فى المنتج (Y_1) فقد تناقص العدد ايضا اثناء فترة الحفظ من 6,5 الى5,2 ثم2,2 فى اليوم السابع على التوالى واختفى فى العينات (Y_2) فى اليوم التاسع. اما في العينات (Y_1)بدون فركتووليجوسكريد فقد ازداد العدد الي 4,6 في اليوم السابع الي ان اختفى في اليوم ال11 من الحفظ.