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YOGHURT ENRICHED WITH CONJUGATED LINOLEIC ACID USING PROBIOTIC BACTERIA BIO PRODUCTION AND ADDITION OF AVOCADO FRUIT

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ABSTRACT

This study was performed to characterize the ability of active Bifidobacteria strains and Avocado to produce conjugated linoleic acid (CLA).Using Bifidobacteria such as B. breve, B. bifidum and B. lactis showed an increase in CLA that convert linoleic acid (LA) to conjugated linoleic acid (CLA).

In first part set yogurt was manufactured using buffalo milk 6% fat, with adding B.breve, B.bifidum and B.lactis. Each of the prepared yoghurt assessed for chemical, microbiological and organoleptic properties when fresh and during refrigerated storage at 6± 1˚C for 14 days.

Results indicated that using probiotic bacteria (B.breve, B. bifidum and B. lactis) was effective in producing conjugated linoleic acid (CLA) in yoghurt treatments during fermentation as compared with the control yoghurt. Contents of probiotic bacteria (*B.breve, B. bifidum and B. lactis*) were decreased in yoghurt treatments during storage, Firmness, Synersiss and pH values were also decreased while T.S, Fat and acidity increased.

In second part stirred yoghurt was made using the same Bifidobacteria species and 10% or 15% avocado fruit. Each of the prepared yoghurt was assessed for chemical, microbiological and organoleptic properties when fresh and during refrigerated storage at 6± 1˚C for 14 days.

Obtained results indicated that adding avocado fruit to probiotic yoghurt highly increased CLA content in all yoghurt treatments tell the end of storage period. Also Bifidobacteria counts gradually increased during storage period.

Keywords: yoghurt, CLA, bifidobacteria bacteria, Avocado.

INTRODUCTION

Conjugated linoleic acid (CLA), refers to a group of positional and geometric isomers of octadecadienoic acid (C18: 2). conjugated double bonds (i.e., the two double bonds are separated by one single bond) in either cis (c) or trans (t) configuration are present predominantly in positions 8 and 10, 9 and 11, 10 and 12, or 11 and 13. The cis9 - trans 11 isomer is the main isomer constituting 90% of the total CLA found in dairy and beef lipids so called rumenic acid. Linoleic acid is converted to CLA by linoleic acid isomers enzyme of rumen bacteria. For these reasons, CLA occurs naturally as a mixture of isomers in dairy foods such as milk and meats derived from ruminant animals. The most abundant isomer of CLA in nature is the cis9 - trans 11 (cis9 - trans 11) isomer. Commercially available CLA is usually a mixture of cis9 - trans11and trans-10, cis-12 (t10c12) isomers with other isomers as minor components. Conjugated linoleic acid isomer mixture and c9t11 and t10c12 isomers alone have been attributed to provide several health benefits that are largely based on animal and in vitro studies.

CLA has been shown to exert various physiological functions such as anti carcinogenic, anti diabetic, anti obesity and prevention of atherosclerosis (Arunabh Bhattacharya, 2006) and (Yanagitab, 2013).The daily intake of CLA is almost 200 mg /day are reviewed by Marialice P.C. Silvestre, (2013) and Microwaving caused a significant decrease in CLA content in milk .The loss was up to 53 % (Walter Bisig., *etal* 2007).

Bifidobacterium species as *Bifidobacterium bifidum LMG 10645*, *Bifidobacterium animalis subsp lactis BB12* and *Bifidobacterium breve LMC520* were used for increasing CLA by microbial conversion of linoleic acid to conjugated linoleic acid and in part 2 using Bifidobacterium species as *Bifidobacterium bifidum* LMG 10645, *Bifidobacterium animalis subsp lactis* BB12 and *Bifidobacterium breve* LMC520 with different ratios from Avocado (*Persea Americana*).

Many researchers investigated the role of common lactic acid bacteria in CLA production. Several strains of food grade micro-organisms have been identified as potential producers of CLA, including strains of *Bifidobacterium, Enterococcus, Lactobacillus, Lactococcus, Propionibacterium* and *Streptococcus*. These cultures may be very important for the food industry, in particular to the dairy industry, as they can be used for the production of traditional and or novel CLA enriched foods through in situ fermentation or used to obtain CLA as a food-ingredient through a biotechnological process. Probiotic or symbiotic dairy foods with potential contents of CLA have been reported by (Rodrigues D, 2011), Silva L I., (2011) and Rodrigues D., (2012).

Avocado, or *Persea American* (P. americana Mill.) is a kind of evergreen fruit plants of the genus Persea family .Avocado contains a variety of essential nutrients as monounsaturated fatty acids (MUFA)-rich fruit oil with 71% MUFA, 13% polyunsaturated fatty acids (PUFA) and 16% saturated fatty acids (SFA). As the avocado fruit ripens, the saturated fat decreases and the monounsaturated fatty acids. Avocados can fit into a heart healthy dietary pattern such as the DASH diet plan USDA and HHS, 2010, Jakobsen *etal*, 2009, de Souza.,*etal*, 2008 and Appel .,*etal* (2005).

Slater.,*etal*., (1975), Moreno .,*etal* (1980) and Lu. et al (2009) showed that the use of avocado dips and spreads as an alternative to more traditional hard, SFA rich spreads or dips can assist in lowering dietary SFA intake Cited from http://www.avocadocentral.com/nutrition/avocado-spread comparison. This study aims to increase CLA content in yogurt using some spps of Bifidobacteria and avocado fruit (*Persea Americana*).

MATERIALS AND METHODS

Materials:

Fresh buffalo's milk: was obtained from Dairy technology Unit, Animal, Production Research Institute, Agric. Res. Center, Ministry of Agriculture.

Yoghurt culture: Mixed culture consisted of *Lactobacillus delbrueckii sub sp. bulgaricus and Streptococcus salivarius sub sp thermophilus* was obtained from Chr. Hansen's Lab A/S Copenhagen, Denmark.

Bifidobacterium strains: *Bifidobacterium bifidum LM 04, Bifidobacterium breve LMC 017* and *Bifidobacterium animalis subsp. Lactis LMG 10645* were obtained from Chr. Hansen Lab., Copenhagen Denmark.

Microbiological media: Microbial media used in this study were ready made using Oxid Division. Oxoid Ltd London.

Persea Americana (Avocado) fruit: was obtained from local market

Methods of analysis

Moisture content: The moisture content was determined gravimetrically according to AOAC (1990) using a Vacuum Oven, Haraeus T5042EK, Germany.

pH: pH values of yoghurt samples were estimated using a digital pH meter (Jenway 3505 pH meter).

Titratable acidity: The acidity was determined by titration following the method described by A.O.A.C. (1990).

Viscosity : Viscosity of yoghurt samples was measured using a Brookfield DV- E viscometer in 100 ml yoghurt at 20˚C.

Fat content: Fat content was determined according to A.O.AC. (2007).

Curd tension (Firmness): Curd tension (Firmness) was measured according to chanderosek hara.,etal (1975).

Syneresis: Syneresis was measured as described by lowerence (1959).

Quantification of CLA production by UV spectroscopy: Total CLA determination was carried out at a wavelength of 233 nm in a Perkin-Elmer spectrophotometer (Lambda650 model, Beaconsfield, UK) with scan program (190–350 nm). Measurements were obtained in triplicate from 2 ml of the lipid extract in hexane placed into quartz cuvettes. In order to verify the suitability of this method, a standard curve was constructed for the absorbance at 233 nm versus CLA (C18:2 c9, t11) concentration (0–30 lg/ml).

Lipid extraction: Lipid isolation from culture media was carried out as a variation of the method described by Alonso, Cuesta, and Gilliland (2003). Briefly: 10 ml of culture media were centrifuged at 7500 rpm, 5 min, and 4 °C. Three ml from the resulting supernatant were added with 6 ml of isopropanol and vortexed for 1 min. Addition of hexane (5 ml) followed which were vortex for 1 min and finally centrifuged at 2000 rpm, 5 min, and 4 centigrade.

Microbiological analysis: Microbiological analysis was done for *B. bifidum, B. breve* and *B.lactis* enumerated (Dave and Shah, 1996) using the modified MRS agar supplemented with 0.05% L. cysteine-HCL. The antibiotic mixture NPNL (Neomycin sulphate, paramonycin sulphate, Nalidixic acid and lithium chloride) as a selective agent was sterilized by filtration through 0.22 µm Millipore filter (Gellman Sci., England) then added to the medium at a rate of 50 ml/L medium just before pouring into the plates. The L. cystein-HCL solution which sterilized by filtration (0.05% final concentration) was

also added using MRS-Salccin agar. The plates were incubated at 37°C for 48 hrs. Total bacterial count was measured as suggested by The American Public Health association APHA (1992).

Preparation of probiotic set yoghurt: Fresh buffalo's milk of 6% fat was heated to 80°C for 20 min, and cooled to 42°C. Such milk was divided into 4 equal portions. The 1st was inoculated with 3% yoghurt culture (control), the 2nd inoculated with3% yoghurt culture and *Bifidobacterium bifidum* (1:1), the 3rd inoculated with with3% yoghurt culture and *Bifidobacterium breve* (1:1), and the 4th inoculated with3% yoghurt culture and *Bifidobacterium lactis* (1:1). The prepared probiotic set yoghurt and their control were assessed for chemical, microbiological and organoleptic properties when fresh and during refrigerated storage at 6± 1 ˚C for 14-day.

Preparation of probiotic stirred yoghurt fortified with Avocado: Fresh buffalo's milk of 6% fat was heated to 80°C for 20 min and cooled to 42°C. Such milk was divided into 9 equal portions. The 1st was inoculated with 3% yoghurt culture, 2 nd inoculated with 3% yoghurt culture with 100 gm Avocado /L milk, 3rd inoculated with3% yoghurt culture + *B.bifidum* (1:1) and 100 gm Avocado /L milk ,the 4 th inoculated with with3% yoghurt culture and Bifidobacterium breve (1:1) + 100 gm Avocado /L milk ,the 5th inoculated with3% yoghurt culture and *B.lactis* (1:1)+ 100 gm Avocado /L milk), the 6th inoculated with3% yoghurt culture +150 gm avocado, the 7th inoculated with3% yoghurt culture and B. *B.Bifidum* (1:1)+ 150 gm Avocado/L milk; the 8th inoculated with with3% yoghurt culture + *B.breve* (1:1) and + 150 gm Avocado /L milk and the 9 th inoculated with 3% yoghurt culture and B. lactis (1:1) + 150 gm Avocado /L milk and stirred .The prepared probiotic yoghurt were assessed for chemical, microbiological and organoleptic properties at fresh and during refrigerated storage at 6± 1 ˚C for 15-day.

Sensory evaluation: All samples were evaluated according to Clark *etal.* (2009)

RESULTS AND DISCUSSION

1-Use of some Bifidobacteria species for Conjugated linoleic acid bio production in set yoghurt

Table 1. Effect of using different Bifidobacteria species on some properties of set yoghurt during cold storage (6±1)

	% Fat		% T.S		Acidity					pH				
reatments	0	14	0	14	0	3	7	10	14	0	3	7	10	14
	----------(Days)----------													
1	6.00	6.03	15.2	16.0	0.63	0.73	0.82	0.91	1.00	4.63	4.54	4.46	4.33	4.21
2	6.00	6.03	15.1	16.0	0.66	0.76	0.88	0.99	1.15	4.54	4.44	4.33	4.25	4.10
3	6.00	6.03	15.1	16.3	0.68	0.80	0.92	1.08	1.21	4.57	4.49	4.34	4.21	3.94
4	6.00	6.03	15.3	16.5	0.64	0.75	0.84	0.94	1.10	4.57	4.47	4.32	4.19	4.06

1- Control yoghurt culture (3 %)
2- Yoghurt culture + *Bifidobacterium bifidum* (3 %) 1:1.
3- Yoghurt culture + Bifidobacterium *breve* (3 %) 1:1.
4- Yoghurt culture + *Bifidobacterium lactis* (3 %) 1:1.

Data presented in table (1) show an increase in fat and total solids (T.S) contents of yoghurt samples after 14 days of cold storage (6±1°c). These increases could be attributed to water evaporation during storage period. Gradual increasing in yoghurt titratable acidity (as lactic acid %) was also observed during cold storage period while pH values of the same samples showed the opposite trend.

Table 2. Conjugated linoleic acid content of set yoghurt treatments fortified with some Bifidobacteria spps during cold storage (6±1)

Treatments	CLA content (mg/ 100 gm fat)							
	0	% Increasing	3	% Increasing	10	% Increasing	14	% Increasing
	----------(Days)----------							
1	0.18	0000	0.23	0000	0.30	0000	0.41	0000
2	0.33	88.00	0.52	127.5	0.64	112.5	0.87	110.0
3	0.37	109.5	0.48	112.7	0.75	148.5	1.02	147.5
4	0.28	60.00	0.44	95.50	0.60	99.6	0.74	77.90

Data tabulated in table 2 clearly show a noticeable increase in fresh yoghurt CLA contents when Bifidobacteria species were incorporated with yoghurt starters comparing to control treatment (1). CLA concentrations of probiotic yoghurt treatments 2, 3 and 4(containing Bifidobacterium species) were nearly the twice. On the other hand gradual increases in CLA content of all yoghurt treatments during cold storage period were observed tell 14 days. The highest CLA content was recorded in treatments (3) samples (*Bifidobacterium breve*) after 14 days of storage (the end of storage period). Samples of treatments (2 and 4) (*Bifidobacterium bifidum* and *Bifidobacterium lactis*) showed less CLA content than samples of treatments (3).Obtained results are in accordance with those found by Kianoush .,*et al* (2014).

Table 3.Changes in some rehological properties of set yoghurt treatments during cold storage (6±1)

Treatments	Firmness \ gm				Synersiss \ ml			
	0	3	10	14	0	3	10	14
	----------(Days)----------							
1	50.6	49.2	45.0	43.0	٢١	٢٠	١٨	١٦
2	48.6	46.6	42.0	39.0	٢٣	٢٣	٢١	١٩
3	45.7	44.0	39.9	37.8	٢٣	٢٥	٢٢	٢٠
4	44.6	42.8	40.0	36.9	٢٤	٢٥	٢٣	٢١

Changes in yoghurt sample firmness and synersiss are presented in table (3). The obtained results show gradual decrease in both firmness and synersiss values tell the end of storage period. In general probiotic yoghurt samples (treatments 2, 3 and 4) showed lower firmness values and higher synersiss volumes as compared with control samples (treatment 1) during storage period. There findings are in agreement with those obtained by Ali., *etal* (2013) and Moayednia (2014).

Table 4.Changes of Bifidobacteria counts in set yoghurt treatments during cold storage (6±1)

Treatments	Changes in Bifidobacteria spps counts (10^7)						
	0	% Decreasing	3	% Decreasing	10	% Decreasing	14
	(Days)						
1	nd	000	nd	000	nd	000	nd
2	196	3.00	189	13.5	169	12.5	171
3	168	8.00	154	11.0	148	14.8	143
4	176	5.80	166	5.50	168	7.30	163

Table 4 showed that *bifidobacterium breve, bifidobacterium bifidum* and *bifidobacterium lactis* count decreased gradually during storage period for 14 days because of increase of acidity, Low oxygen content and low redox potentials have been cited as important factors for the viability of bifidobacteria during storage of fermented milk products (Brunner .,*etal* 1993a and 1993b).

Table 5. Sensory evaluation of fresh set yoghurt treatments

Treatment	Flavor(20)	Color(10)	Texture(10)	Sensory evaluation(40)
1	19	10	9	38
2	18	10	8	36
3	17	10	8	35
4	16	10	8	34

Sensory evaluation (total score 40) of fresh yoghurt samples are shown in table (5). Tabulated data show that control samples gained the highest score reaching 38, while probiotic yoghurt samples gained less score specially treatment (4) (with *B.lactis*).

Π-Enrichment of probiotic stirred yoghurt with avocado fruit

Table (6) Effect of adding avocado fruit on some properties of probiotic stirred yoghurt during cold storage (6±1)

reatments	% Fat		% T.S		Acidity					pH				
	0	14	0	14	0	3	7	10	14	0	3	7	10	14
	(Days)													
1	6.00	6.10	15.2	16.0	0.64	0.73	0.82	0.92	1.00	4.62	4.54	4.46	4.33	4.21
2	6.90	7.20	20.6	21.7	0.69	0.79	0.88	0.96	1.10	4.42	4.31	4.22	4.11	4.01
3	6.90	7.20	20.6	21.6	0.71	0.83	0.90	1.07	1.21	4.35	4.21	4.11	4.00	3.87
4	6.90	7.20	20.6	21.7	0.73	0.88	0.99	1.13	1.28	4.32	4.17	4.03	3.89	3.78
5	6.90	7.10	20.8	21.9	0.69	0.82	0.90	1.00	1.17	4.37	4.28	4.10	3.97	3.86
6	7.30	7.60	20.8	21.7	0.71	0.84	0.92	1.11	1.24	4.27	4.18	4.07	3.91	3.88
7	7.30	7.60	20.9	21.8	0.73	0.88	1.00	1.16	1.32	4.36	4.29	4.16	4.02	3.92
8	7.30	7.60	20.692	21.668	0.74	0.90	1.02	1.21	1.37	4.30	4.18	4.05	3.88	3.71
9	7.30	7.70	20.686	21.635	0.72	0.88	0.96	1.14	1.26	4.34	4.20	4.10	3.91	3.81

1-Control yoghurt culture (3 %).
2-Yoghurt culture (3 %) +100 gm avocado/1 liter milk.
3-Yoghurt culture + *bifidobacterium bifidum* (1:1) +100 gm avocado / 1 liter milk.
4-Yoghurt culture + *bifidobacterium breve* (1:1) +100 gm avocado / 1 liter milk.
5-Yoghurt culture + *bifidobacterium lactis* (1:1) +100 gm avocado / 1 liter milk.
6- Yoghurt culture (3%) +150 gm avocado / 1 liter milk.
7- Yoghurt culture + *bifidobacterium bifidum* 1:1+ 150 gm avocado / 1 liter milk.
8-Yoghurt culture + *bifidobacterium breve* (1:1) +150 gm avocado / 1 liter milk.
9- Yoghurt culture + *bifidobacterium lactis* (1:1) +150 gm avocado / 1 liter milk.

Effect of adding avocado fruit (10 or 15%) on fat, T.S, acidity and pH of probiotic yoghurt samples during cold storage is shown in table (6). Tabulated data showed obvious increase in fat and T.S content due to avocado addition in fresh yoghurt samples. During cold storage fat and T.S content were increased as mentioned before in table (1) due to water evaporation. Titratable acidity of yoghurt sample gradually increased through out storage period while pH values decreased tell the end of storage. Addition of avocado fruit clearly affected titratable acidity and pH of fresh yoghurt samples.

Table7.Content of CLA in stirred yoghurt fortified with some Bifidobacteria spps and different ratios of avocado during cold storage (6±1)

Treatments	CLA content (mg/ 100 gm fat)							
	0	% Increasing	3	% Increasing	10	% Increasing	14	% Increasing
	----------(Days)----------							
1	0.18	0000	0.28	000	0.30	000	0.41	000
2	1.10	513.4	1.22	437	1.82	500	2.56	518
3	1.18	562.9	1.46	542	2.40	691	2.80	575
4	1.67	838.2	1.70	649	2.72	797	3.09	647
5	1.04	485.3	1.20	430.	1.85	510	2.31	458
6	1.37	671.3	1.75	672.6	2.56	744	2.86	591
7	1.57	783.7	1.99	777.9	2.83	834	3.33	705
8	1.93	987.0	2.35	937.4	3.25	972	3.64	780
9	1.40	687	1.80	691	2.62	764	3.10	650

Results in table (7) indicated that CLA contents gradually increased during storage period. Also these results obviously showed significant increase in CLA content when avocado fruit was added to yoghurt samples. In other hand CLA content increasing was positively correlated with avocado concentration used. On the other hand CLA content increasing was positively correlated with avocado concentration used.

Table 8.changes in viscosity of stirred Yoghurt treatments fortified with avocado during cold storage (6±1)

Treatment	Viscosity(cp)			
	0	3	10	14
	----------(Days)----------			
1	6000	6600	8800	9800
2	9000	9800	12400	13200
3	8000	9000	11700	12400
4	8500	9300	11800	12300
5	8700	9700	12000	12800
6	10100	11000	11700	13700
7	9700	10300	11400	12900
8	9700	10800	11800	12900
9	10000	11000	12300	13400

In general CLA increase could be attributed to avocado addition and conversion of LA to CLA during biohydrogenation by bifidobacteria species. (Milutal, 1992). The highest CLA content was recorded in treatments (9) sample (*Bifidobacterium breve* + 150 gm avocado fruit) after 14 days of storage (the end of storage period). Samples of other treatments showed less CLA content than samples of treatments (9).

Data presented in table (8) show gradual increases in viscosity values of Yoghurt treatments during storage period. Viscosity increase could be due to acidity development and avocado fortification. (Davenport,2013).

Table 9. Changes of Bifidobacteria counts in stirred yoghurt treatments fortified with avocado during cold storage (6±1)

Treatments	Changes in Bifidobacteria spps counts(10^{7})						
	0	% Increasing	3	% Increasing	10	% Increasing	14
	----------(Days)----------						
1	nd		nd		nd		nd
2	nd		nd		nd		nd
3	364	19.7	436	35.7	494	44.0	525
4	240	29.0	310	67.0	401	113	512
5	286	15.7	331	51.0	432	92.3	550
6	nd		nd		nd		nd
7	389	28.0	498	58.0	616	82.0	708
8	261	38.0	361	73.0	452	120	575
9	314	20.0	377	51.0	475	92.0	605

Table 9 showed that *Bifidobacterium breve*, *Bifidobacterium bifidum* and *Bifidobacterium lactis* counts gradually increased during storage period for 14 days due to avocado addition. The highest increase of Bifidobacterium counts was recorded with *Bifidobacterium bifidum* treatments as it was previously mentioned (table4). Increase of probiotic bacterial counts could be ascribed by the simultaneous presence of inhibitory compounds such as antibacterial poly phenolics (Ripa.,*etal* ,2009).

Results in table (10) showed sensory evaluation of fresh probiotic yoghurt treatments fortified with avocado fruit. Almost no differences were recorded between all treatments except control sample (1).

Table 10. Sensory evaluation of fresh stirred yoghurt treatments fortified with avocado

Treatments	Flavor(20)	Color(10)	Texture(10)	Sensory evaluation(40)
1	19	10	9	38
2	15	8	9	32
3	15	8	9	32
4	15	8	9	32
5	15	8	9	32
6	16	7	9	32
7	16	7	9	32
8	15	7	9	31
9	16	7	9	32

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اليوجورت المدعم بحمض اللينوليك المرتبط باستخدام الانتاج الحيوى لبكتريا البروبايوتك بالاضافه الى فاكهة الافوكادو

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أجريت هذه الدراسة لمعرفة قدرة سلالات البفيدوبكتيريا النشطة والأفوكادو لإنتاج حمض اللينوليك المرتبط (CLA) وقد اظهرت البفيدوبكتيريا B. Breve, B. bifidum and B. lactis زيادة في CLA حيث يتم تحويل حمض اللينوليك (LA) إلى حمض اللينوليك المرتبط (CLA).

فى الجزء الاول تم تصنيع اليوجورت من لبن جاموسى ٦% دهن يحتوى على B.breve, B.bifidum and B.lactis حيث وتم تقييم اليوجورت الناتج كيميائيا و ميكروبيولوجيا و حسيا و ذلك لليوجورت الطازج وخلال فترة التخزين على ٦ ±١ درجه مئويه لمدة ١٤ يوم.

و قد اوضحت النتائج ان استخدام بكتريا البروبايوتك B.breve, B. bifidum and B. lactis كان لها تاثير على زيادة انتاج حمض اللينوليك المرتبط اثناء التخمر مقارنة بالكنترول .

وقد انخفضت اعداد بكتريا البروبايوتك B.breve, B. bifidum and B. lactis كما انخفض معدل طرد الشرش و معدل صلابة الخثره و قيم الرقم الهيدروجينى pH بينما ارتفعت قيم كل من الجوامد الكليه و الدهن والحموضه.

وفى الجزء الثانى تم تصنيع اليوجورت باستخدام نفس السلالات البكتيريه مع اضافه نسب ١٠او١٥ % من فاكهة الافوكادو و تم تقييم الناتج كيميائيا و ميكروبيولوجيا و حسيا و ذلك لليوجورت الطازج وخلال فترة التخزين على ٦ ±١ درجه مئويه لمدة ١٤ يوم.

وادت اضافة الافوكادو لليوجورت المقلب المدعم ببكتريا البروبايوتك الى زيادة نسبة حمض اللينوليك المرتبط فى جميع المعاملات حتى نهاية مدة التخزين كما ادت الى زياده اعداد بكتريا البروبايوتك خلال مدة التخزين.