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USING MORINGA LEAVES POWDER IN PRODUCTION OF PROBIOTIC YOGHURT

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ABSTRACT

Moringa leaves powder was added during the manufacture of probiotic yoghurt at level (0.5%) before pasteurization. Three probiotic yoghurt treatments were prepared as follows: yoghurt without moringa served as control (T_1), yoghurt + moringa (T_2) and yoghurt + moringa + 10% mango pulp (T_3). Yoghurt were inoculated with 2% lactic acid bacteria (LAB) and 2% *L. acidophilus* and incubated at 42°C until complete coagulation (pH 4.8), then stored at 5°C up to 14 days. Chemical, microbiological and sensory properties of the produced yoghurt were carried out. The level of 0.5% moringa was found to be the best ratio. The results showed that pH values and moisture content (%) decreased during storage period for all treatments, while the values of titratable acidity, total solids (%), protein content (%), fat content (%), antioxidant activity and total phenolic content were increased and the treatments (T_2 and T_3) had values greater than control yoghurt (T_1). Microbiological analysis indicated that the addition of 0.5% moringa leaves powder and 0.5% moringa leaves powder +10% mango pulp stimulate the growth of LAB and probiotic culture (*L. acidophilus*). On the other hand Yeasts and moulds, Coliform and Sporeforming bacteria were not detected in all treatments up to the end of storage period. Moreover the addition of 0.5% moringa leaves powder + 10% mango pulp increased the acceptability of product more than the addition of moringa alone up to the 14th day of storage at 5°C.

Kew words: Moringa, production, yoghurt.

INTRODUCTION

Yoghurt is a coagulated dairy product obtained by the lactic acid fermentation of milk by bacteria *i.e. Streptococcus thermopiles* (ST), *Lactobacillus delbrueckii* ssp. *bulgaricus* (LB) **(Fadela *et al.,* 2009).** Addition of these two cultures resulted in acidification of milk and produce of aromatic compounds **(Sahan *et al.,* 2008)**. Although these microflora have been found to be valuable for human as they help in maintaining health and nutrition. Also efforts have been placed on developing yoghurt containing probiotic cultures like *Lactobacillus acidophilus* (LA) and *B. bifidus* (BB) **(Vinderola and Reinheimer, 2000)**. Probiotic cultures are live microbial food ingredients that are beneficial for human health **(Salminen *et al.,* 1999)**, which includes improvement of intestinal microbial balance which results in the inhibition of bacterial pathogens, reducing the risk of colon cancer, in the inhibition of bacterial pathogens, reducing the risk of colon cancer, improving the immune system, lowering serum cholesterol levels **(Saarela *et al.,* 2002)**, alleviation of lactose intolerance and nutritional enhancement **(Alizadeh and Ehsani, 2008).**

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Mango (*Mangifera indica* L.) is a seasonal fruit grows in tropical regions and is regarded as one of the most important fruits of Asia.

The nutritional importance of mango is mainly due to its high amounts of b-carotene, a carotenoid which provides various health benefits, including pro-vitamin A and antioxidant activity *(***Harnkarnsujarit and Charoenrein, 2011**). Mango contains a variety of phytochemicals and nutrients. The fruit pulp is high in prebiotic dietary fiber, vitamin C, diverse polyphenols and provitamin a carotenoids (**Ajila and Prasada Rao, 2008**).

Moringa oleifera is referred to as a "Miracle tree" or "Wonder tree" (**Kasolo *et al.,* 2010)** of significant socio economic importance because of its several nutritional, pharmacological (**Caceres *et al.,* 1991**) and industrial application (**Makkar and Becker, 1996).**

The leaves of this plant contain high amount of vitamin B complex, calcium, potassium, iron and protein. Also, they contain all of the essential amino acids in good proportion (**Mishra *et al.,* 2012**).

Moringa oleifera leaves are active against the growth of bacteria such as*: E. coli, S. arous, P. aeruginosa and B. cereus* as these organisms range from pathogenic and oxygenic organism liable to cause food borne illnesses and food spoilage. It can be used as evaluable drug in the treatment of infections caused *by E. coli* and *P. aeruginosa* (**Abalaka *et al.,* 2012).**

MATERIALS AND METHODS

Materials

Fresh cow's milk was obtained from the herd of Badwy farm of El-Arish, Egypt. Average chemical composition of milk (3% fat, 3.35% protein, 12.6% T.S) were determined according to **AOAC (2011)**.

Skim milk powder (96% TS, product of Dairy America TM) USA, was obtained from the local market.

Direct Vat Starter (DVS) yoghurt culture was obtained from CHR-Hansen's laboratorie, Denmark, under commercial name type (FD-DVS-YC-X11) containing *Streptococcus thermophiles* and *Lactobacillus delbrueckii* ssp. *Bulgaricus.*

Probiotic bacteria strain *Lactobacillus acidophilus* (DSM20384) was obtained from Egyptian Microbial Culture Collection (EMCC) at Cairo Microbiological Resources Center (Cairo MIRCEN), Faculty of Agriculture, Ain Shams University.

Mango (*Mangifera indica*) fruit and sugar were obtained from local market of El-Arish, Egypt.

Moringa oleifera leaves was obtained from Cautia farm of North Sinai, Egypt.

Methods

Preparation of Additions

Preparation of mango pulp

Mango pulp (0.27% fat, 0.51% protein, 81.3% T.S) was obtained manually after thorough washing and peeling of the skin and blended to get smooth and then pasteurized at 90°C for 10 min according to the procedure mentioned by **Vijayalakshmi *et al.* (2009)**.

Preparation of moringa leaves powder

The collected leaves were spread on a clean curtain cloth and kept at room temp.

The selected room for shade drying was well ventilated by natural current of air. The leaves took about six to seven days to dry completely and became crispy and brittle to touch then blended in a blender to get powder according to the procedure mentioned by **Delong (2003)**.

Preparation of probiotic culture

Strain *Lactobacillus acidophilus* (DSM 20384) was activated in MRS broth according to **De Man *et al.* (1960)**.

Manufacture of Yoghurt

Yoghurt was made from standardized cow's milk according to **Tamime and Robinson (1999)** as shown in diagram (A).

Diagram (A) – Manufacure of yoghurt

Fresh cow milk (3% fat, 3.35% protein, 12.6% TS).

Methods of Analysis

Yoghurt samples were analyzed chmically, microbiologicaly and organoleptically when fresh and after 7 and 14 days of storage at 5°C.

Chemical Analysis

pH values were measured using Jenway pH meter with Jenway spear electrode No: 29010 (Jenway limited Gransmore Green, Felsted, Dunmow, England).

Titratable acidity, total solids, total protein and fat were determined according to the method described by **AOAC (2011)** .Moisture content was calculated using the regular equation as follows:

Moisture (%) = 100 – Total solids

Measurement of antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) inhibition **assay** was carried out according to methods described by **Li *et al.* (2009)**. Total phenolic content (TPC) of the previously prepared yoghurt samples were determined using the Folin- Ciocalteau by method described by **Li *et al.* (2009)**.

Microbiological analysis

Preparation of all samples for microbiological examination was carried out as described by **Frazier and Foster (1961)**.

Lactobacillus acidophilus and *Lactobacillus delbrueckii* ssp. *Bulgaricus* were determined using MRS agar medium as described by **De Man *et al.* (1960)**.

Streptococcus thermophiles was determined by using M17 selective medium as described by **Krusch *et al.* (1987)**. Plates were incubated at 37°C for 48hr.

Moulds and Yeasts Count

Were determined on oxytetracycline glucose yeast extract agar medium as suggested by **Harrigan and Mcconce, (1966).** Plates were incubated at 25°C for 3 days.

Coliform group

Were determined according to the **American Public Health Association (1978)**. Appropriate dilutions of samples were plated on Mac Conk's agar medium and incubated at 37°C for 48hr.

Organoleptic properties

Organoleptic properties of yoghurt samples were evaluated according to **Tamime and Robinson (1999)**.

RESULTS AND DISCUSSION

Chemical Analysis of Yoghurt

Based on the results presented in Table 1. Generally, pH of all yoghurt samples decreased during storage up to 14 days. This phenomena was due to the growth of lactic acid bacteria and the production of lactic acid, which was due to the especial synergistic effect between *Lac.* spp and *Strep.* spp. (**Yousef *et al.*, 2013**).

Also, there were slightly differences in pH values between control yoghurt and treated yoghurts during the storage period. These results were in agreement with those obtained by **Vijayalakshmi *et al.* (2009)**.

It was clear from Table 1 that acidity values of all treatments increased during the progress of storage period.

Moreover, there were slightly differences in acidity values between control yoghurt and treated yoghurts during the storage period **(Lamoureux *et al.*, 2002)** the increase in acidity attributed to the decrease in lactose content and post acidification

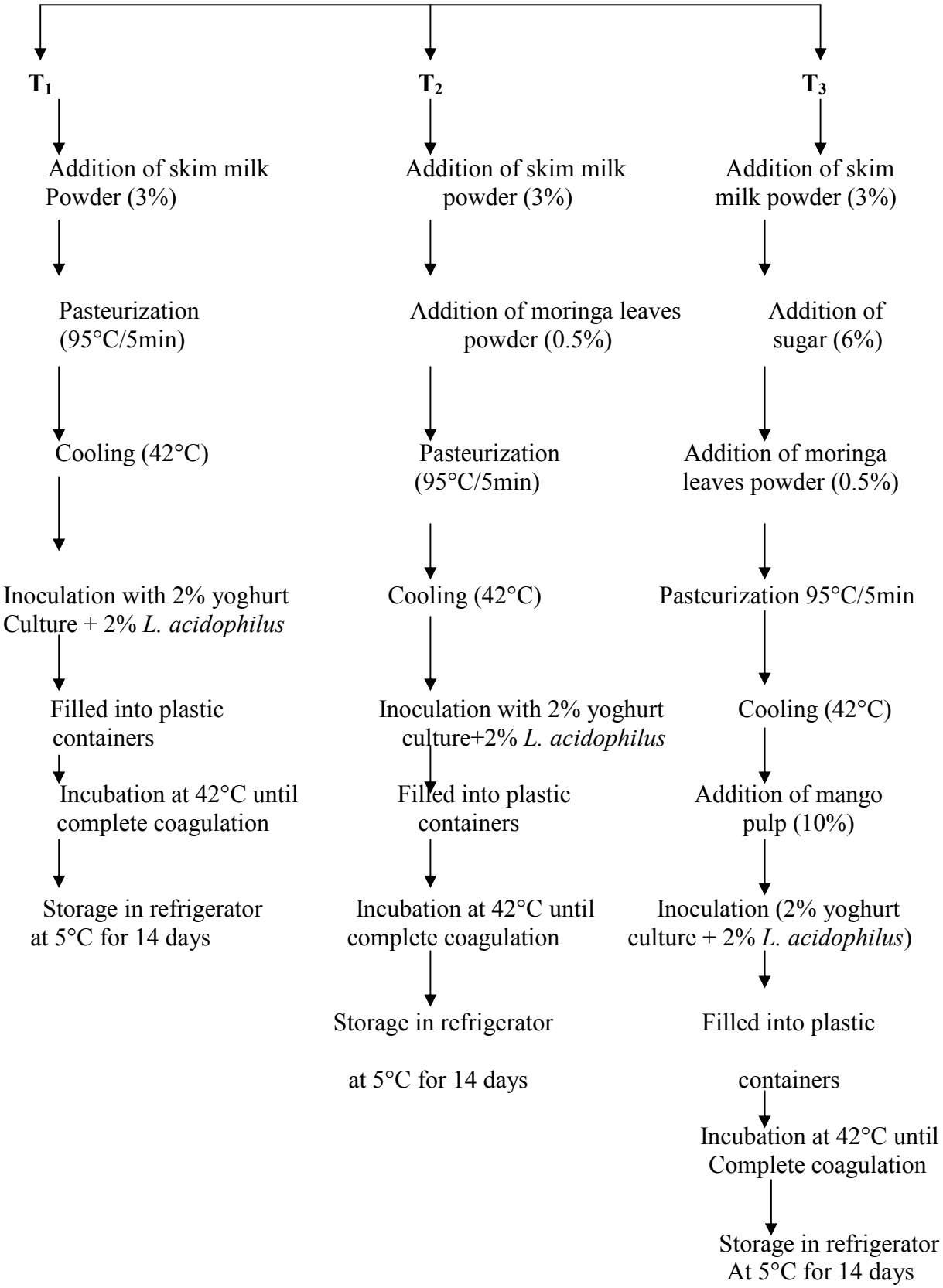
T1
T2
T3
Addition of skim milk Powder (3%)
Addition of skim milk powder (3%)
Addition of skim milk powder (3%)
Pasteurization (95°C/5min)
Addition of moringa leaves powder (0.5%)
Addition of sugar (6%)
Cooling (42°C)
Pasteurization (95°C/5min)
Addition of moringa leaves powder (0.5%)
Inoculation with 2% yoghurt Culture + 2% L. acidophilus
Cooling (42°C)
Pasteurization 95°C/5min
Filled into plastic containers
Inoculation with 2% yoghurt culture+2% L. acidophilus
Cooling (42°C)
Incubation at 42°C until complete coagulation
Filled into plastic containers
Addition of mango pulp (10%)
Storage in refrigerator at 5°C for 14 days
Incubation at 42°C until complete coagulation
Inoculation (2% yoghurt culture + 2% L. acidophilus)
Storage in refrigerator at 5°C for 14 days
Filled into plastic containers
Incubation at 42°C until Complete coagulation
Storage in refrigerator At 5°C for 14 days

Table (1): pH and Acidity values of yoghurt fortified with moringa during storage up to 14 days at 5°C

Parameter	Storage (day)	Treatment		
		T_1	T_2	T_3
pH	Fresh	4.60	4.50	4.50
	7	4.18	4.13	4.13
	14	4.14	4.07	4.06
Acidity (%)	Fresh	0.6	0.7	0.8
	7	0.8	0.9	0.9
	14	0.9	0.9	1.0

T_1: control (milk) T_2: milk + moringa . T_3: milk + moringa + mango + sugar.

especially by *L. delbreuckii* ssp. *bulgaricus* during the storage period. It was clear from Table 2 that the T.S of probiotic yoghurt slightly increased in all treatments during storage and the control treatment was lower than the others allover the storage period.

These results are similar to the values obtained by **Hashim (2007)**. Moreover, the increase in total solids content during storage period attributed to the loss of moisture **(Tamime, 1978)**. Also the protein content of all treatments gradually increased during storage and the control treatment was lower than the others allover the storage period. These results were in agreement with those obtained by **Salem *et al.* (2013)**.

It was clear from Table 2 that the fat content of all treatments were slightly increased gradually during the progress of storage and the control treatment was lower than the others as storage period proceeded.

Increasing fat content in all treatments during storage may be due to the loss of moisture. These results are in agreement with those obtained by **Ismail *et al.* (2006)**.

It is clear from this Table 2 that the moisture content of all treatments decreased gradually during the progress of storage. The values of control treatment was higher than the other treatments during storage period.

The decrease in moisture contents of all treatments allover the storage period was probably due to the increase in total solid values.

Evaluation of Antioxidant activity (%) and Total phenolic content (TPC)

It is clear from Table 3 that the antioxidant activity in fresh yoghurt made with moringa (T_2) exhibited higher significant scavenging activity followed by yoghurt made with moringa + mango (T_3) while the plain yoghurt was found to have a lower scavenging effect. High potential of antioxidant activity of treatments may be due to that they are rich in photochemical contents, which possessed high antioxidant.

On the other hand, DPPH radical scavenging activity of all treatments dropped at the 7^{th} and the 14^{th} days of storage period. Data presented in Table 3 show that the total phenolic content (TPC) of yoghurt fortified with moringa and moringa + mango were significant higher than plain yoghurt. Moreover, at the 7^{th} day of storage period the TPC of all samples decreased significantly and also at the 14^{th} day of storage this may be due to the decreased in pH values throughout storage period.

Table (2): Chemical analysis of yoghurt fortified with moringa during storage up to 14 days at 5°C

Chemical analysis (%)	Storage (day)	Treatment		
		T_1	T_2	T_3
Total solids	Fresh	13.2	14.6	18.4
	7	14.3	15.7	19.1
	14	15.9	16.8	20.5
Protein	Fresh	3.5	3.9	4.0
	7	3.6	4.2	4.3
	14	3.8	4.5	4.8
Fat	Fresh	3.2	3.3	3.4
	7	3.3	3.5	3.6
	14	3.4	3.6	3.7
Moisture	Fresh	86.8	85.4	81.6
	7	85.7	84.3	80.9
	14	84.1	83.2	79.5

T_1: control (milk) T_2: milk + moringa . T_3: milk + moringa + mango + sugar.

Table (3): Antioxidant activity (%) and Total phenolic content (TPC) of yoghurt fortified with moringa during storage up to 14 days at 5°C

Parameter	Storage (day)	Treatment		
		T_1	T_2	T_3
Antioxidant activity (%)	Fresh	67.34	93.46	87.83
	7	43.85	84.63	75.39
	14	37.64	77.56	63.84
TPC (mg Gallic acid/100 gm.sample)	Fresh	9.86	15.89	14.71
	7	8.73	15.04	14.13
	14	7.93	13.85	12.96

T_1: control (milk) T_2: milk + moringa . T_3: milk + moringa + mango + sugar.

Microbiological Analysis

Results cleared that there were an obvious differences between treatments of bio-yoghurt in the viable numbers of *S. thermopiles* and *L. bulgaricus* when fresh and during storage period. The counts were increased up to the 7th day then decreased during the progress of storage. The highest count obtained was of yoghurt made with moringa (T_2) followed by yoghurt made with moringa + mango (T_3) while the lowest count was in control. Moringa alone stimulate the growth of both (*Lactobacillus acidophilus and Lactobacillus bulgaricus)* more than moringa + mango. The decline in bacterial counts may be due to the decreasing in the pH value of yoghurt **(Yannawa *et al.*, 2014)**.

These results are in agreement with those obtained by **Vijayalakshmi *et al.* (2009), Sharareh *et al.* (2015), Salem *et al.* (2013)** moreover **Van Tienen *et al.* (2011)** suggested that the growth of the probiotics in *M. oleifera*-supplemented yoghurt was found to have a growth-enhancing effect.

It was clear from this Table 4 that the count of *S.thermophilus* increased gradually up to the 7th day of storage then decreased during the progress of storage. The highest count obtained was in yoghurt made with moringa (T_2) while the lowest count obtained was in control (T_1). These results are in agreement with those obtained by **Vijayalakshmi *et al.* (2009), VanTienen *et al.* (2011), Salem *et al.* (2013) and Sharareh *et al.* (2015)**.

It was clear from the same Table that yeast and mould and coliform group were not detected in all treatments allover the storage period.

Organoleptic properties

Data in Table 5 shows that the total scores of sensory evaluation of all treatments were gradually decreased during storage. This may be due to the increase in the acidity which affect the rheological properties. In general, the values of total sensory evaluation were in the following desending order $T_3 > T_1 > T_2$. These results are in agreement with those obtained by **Madhu *et al.* (2012) and Sharareh *et al.* (2015)**.

Table (4): Microbiological analysis of yoghurt fortified with moringa during storage up to 14 days at 5°C

Type of culture	Storage (day)	Treatments (log (cfu/ml)		
		T_1	T_2	T_3
***L. acidophilus, L. bulgaricus* count**	**Fresh**	8.32	8.45	8.44
	7	9.42	10.30	10.13
	14	9.30	9.93	9.03
***S. thermophilus* count**	**Fresh**	8.10	8.12	8.11
	7	8.93	10.15	10.04
	14	7.90	9.01	8.93
Yeast & Mould and Coliform count	**Fresh**	ND	ND	ND
	7	ND	ND	ND
	14	ND	ND	ND

T_1: control (milk) T_2: milk + moringa . T_3: milk + moringa + mango + sugar.

Table (5): Organoleptic properties of yoghurt fortified with moringa during storage up to 14 days at 5°C

Sensory parameter	Storage (day)	Treatment		
		T_1	T_2	T_3
Appearance (5 marks)	Fresh	4.95	4.87	4.80
	7	4.95	4.87	4.80
	14	4.90	4.85	4.75
Body & Texture (5 marks)	Fresh	4.93	4.90	4.85
	7	4.93	4.90	4.85
	14	4.90	4.85	4.80
Flavor (10 marks)	Fresh	8.85	8.0	9.5
	7	8.85	8.0	9.5
	14	8.80	7.5	9.3
Total acceptance (20 marks)	Fresh	18.7	17.77	19.15
	7	18.7	17.77	19.15
	14	18.6	17.20	18.85

T_1: control (milk) T_2: milk + moringa. T_3: milk + moringa + mango + sugar.

Conclusion

Finally it was concluded, from the previous data that, the addition of 0.5% moringa leaves powder and 0.5% moringa leaves powder +10% mango pulp in the manufacture of pro-bioticyoghurt stimulate the growth of LAB and probiotic culture (*L. acidophilus*) so increased the nutritional value of yoghurt. Also all treatments had a high positive effect on total phenolic contents and its antioxidant properties. Moreover the addition of 0.5% moringa leaves powder +10% mango pulp in the manufacture of yoghurt increased the acceptability of product more than the addition of moringa alone up to the 14th day of storage at 5°C.

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الملخص العربى

استخدام مسحوق أوراق المورنجا في إنتاج زبادي وظيفي

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تم تصنيع الزبادي باستخدام لبن بقرى تم تقسيمه إلى ثلاثة أجزاء متساوية كل جزء تم معاملته كالاتي: المعاملة الأولى: تم تسخين اللبن على ٩٥°م لمدة خمس دقائق ثم تم تبريده لدرجة حرارة ٤٢°م لتلقيح بكتريا البادئ المكونة من ٢% (١:١) من كلا من *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophiles* و ٢% من بكتريا *Lactobacillus acidophilus* ثم التحضين على درجة حرارة ٤٢°م حتى تمام التماسك وهذه المعاملة هى الكنترول لكل المعاملات وفى المعاملة الثانية تم إضافة ٠,٥% من مطحون اوراق المورنجا للبن قبل البسترة وباقى الخطوات كما فى المعاملة الأولى وفى المعاملة الثالثة تم إضافة ٦% سكر و ٠,٥% من مطحون أوراق المورنجا للبن قبل البسترة وإضافة ١٠% من لب المانجو للبن بعد البسترة وقبل التلقيح بالبكتريا وباقى الخطوات كما فى المعاملة الأولى الزبادي الناتج تم تحليله فى اليوم الأول وكذلك أثناء التخزين على ٥°م بعد ١٤,٧ يوم من التخزين حيث تم عمل اختبارات كيميائية وميكروبيولوجية وحسية للمنتج ويمكن تلخيص النتائج كما يلى:

لوحظ انخفاض فى قيم الpH لجميع المعاملات وكانت المعاملة الكنترول اعلى بنسبة بسيطة عن باقى المعاملات من اليوم الأول وحتى اليوم ١٤ من فترة التخزين وازدادت قيم الحموضة اثناء فترة التخزين وكانت المعاملة الثالثة (زبادى بالمورنجا والمانجو) اعلى من باقى المعاملات بينما كانت المعاملة الكنترول اقلها من اليوم الاول وحتى ١٤يوم من فترة التخزين كما ازدادت قيم المادة الصلبة لجميع المعاملات اثناء فترة التخزين وكان الزبادى المدعم بالمانجو والمورنجا اعلى من باقى المعاملات بينما كانت المعاملة الكنترول اقلها من اليوم الأول وحتى نهاية فترة التخزين و ازدادت نسبة البروتين تدريجيا لجميع المعاملات اثناء فترة التخزين وكان الزبادى المدعم بالمورنجا والمانجو اعلى من باقى المعاملات يليه الزبادى المدعم بالمورنجا بينما كانت المعاملة الكنترول اقلها بدءاً من اليوم الاول وحتى انتهاء فترة التخزين و ازدادت نسبة الدهن معنويا لجميع المعاملات اثناء فترة التخزين وكانت المعاملة الكنترول أقل من باقى المعاملات خلال فترة التخزين وانخفضت الرطوبة تدريجيا لجميع المعاملات اثناء فترة التخزين وكانت المعاملة الكنترول اعلى فى محتوى الرطوبة من باقى المعاملات بينما كان الزبادى المدعم بالمورنجا والمانجو اقلها فى محتوى الرطوبة بدءاً من اليوم الاول وحتى نهاية فترة التخزين كما احتوى الزبادى المدعم بالمورنجا على قيم مرتفعة من النشاط المضاد للأكسدة ثم يعقبه الزبادى المدعم بالمورنجا والمانجو وكانت المعاملة الكنترول اقل المعاملات فى قيم النشاط المضاد للاكسدة, وقد لوحظ انخفاض فى قيم النشاط المضاد للاكسدة فى اليوم السابع من التخزين, وقد استمر هذا الانخفاض حتى اليوم ١٤من فترة التخزين والمحتوى الكلى للفينولات للزبادى المدعم بالمورنجا اعلى قيماً من الكنترول, كما لوحظ انه فى اليوم الاول سجلت اعلى قيم للمحتوى الكلى للفينولات فى الزبادى المدعم بالمورنجا ثم الزبادى المدعم بالمورنجا والمانجو بينما سجلت اقل قيمة للمعاملة الكنترول، أما خلال اليوم السابع من فترة التخزين حدث انخفاضا لقيم المحتوى الكلى للفينولات لجميع المعاملات واستمر هذا الانخفاض حتى اليوم ١٤من فترة التخزين، إضافة كلا من المورنجا والمورنجا مع المانجو الى الزبادى أدى إلى تحفيز نمو كلا من *L. bulgaricus, L. acidophilus* and *S. thermopiles* حيث كان للمورنجا بمفردها تاثيرا محفزا لنمو هذه البكتريا اكثر من المورنجا مع المانجو، وقد سجلت اعلى قيم لأعداد البكتريا في اليوم السابع من التخزين بينما حدث انخفاض لا عداد البكتريا فى اليوم الرابع عشر من فترة التخزين بينما تلاشى تماما المحتوى الميكروبى من الخمائر والفطريات ومن بكتريا الكوليفورم فى جميع المعاملات حتى نهاية فترة التخزين و حصل الزبادى الكنترول على اعلى درجة تحكيم فى المظهر خلال اليوم الاول واليوم السابع من التخزين ثم يعقبه الزبادى المدعم بالمورنجا بينما حصل الزبادى المدعم بالمورنجا والمانجو على اقل درجة تحكيم فى المظهر، وفى اليوم الرابع عشر من التخزين قلت درجات التحكيم لجميع المعاملات حصل الزبادى الكنترول على اعلى درجات تحكيم فى القوام والتماسك في اليوم الاول واليوم السابع من التخزين بينما حصل الزبادى المدعم بالمورنجا والمانجو على اقل درجة تحكيم فى القوام والتماسك، وفى اليوم الرابع عشر من التخزين قلت درجات التحكيم نسبيا وحصل الزبادي المدعم بالمورنجا والمانجو على اعلى درجة تحكيم فى النكهة ثم يعقبه الزبادى الكنترول فى اليوم الاول واليوم السابع من فترة التخزين بينما حصل الزبادى المدعم بالمورنجا على اقل درجة تحكيم فى النكهة، وفى اليوم الرابع عشر من فترة التخزين قلت درجات التحكيم نسبيا حصل الزبادى المدعم بالمورنجا والمانجو على أعلى درجات القبول العام ثم يعقبه الزبادى الكنترول من اليوم الاول وحتى ١٤ يوم من فترة التخزين بينما حصل الزبادى المدعم بالمورنجا على اقل درجة قبول عام عن باقى المعاملات خلال فترة التخزين ومن من النتائج المتحصل عليها يمكن القول أن تصنيع الزبادى وتدعيمه ب (٠,٥% مطحون أوراق مورنجا – ٠,٥% مطحون أوراق مورنجا + ١٠% لب مانجو) أدى إلى تحفيز نمو بادئ الزبادى وايضا ال probiotic culture (*L. acidophilus*) و كان لها تاثيرا ايجابيا عاليا فى المحتوى الكلى للفينولات والنشاط المضاد للاكسدة فى الزبادى الناتج, وعلاوة على ذلك فان تدعيم الزبادى ب (٠,٥% مطحون أوراق المورنجا + ١٠% مانجو) أدى إلى تحسين القبول العام للزبادى اثناء فترة التخزين حتى ١٤ يوم على درجة حرارة ٥°م.

الكلمات الإسترشادية: المورنجا، إنتاج، زبادى.
