

EFFECT OF DIFFERENT BACTERIAL STRAINS ON LIKE-LABNEH PRODUCTS FROM BUFFALEO'S MILK AND HULL-LESS BARLEY

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ABSTRACT

Like-labneh products were prepared with buffaloes' milk contained 14 % total solids, barley flour 10 % and butter milk 6 %. The mixture was inoculated using five strains to give five treatments. Treatment I contained 3.0% yoghurt culture, treatment II contained 1.5%Bifidobacterium lactis and *Lactobacillus acidophilus*, treatment III contained 3.0 % ABT cultures, treatment IV contained 1.5 % from treatment II strains plus 0.5 % glucon delta lacton and treatment V contained 1.5 % ABT plus 0.5 % glucon delta lacton. The chemically, microbiologically and organoleptically were determined in like-labneh products at different storage periods zero time, 4, 8, 18 and 30 days at 5°C in refrigerator. The results showed that the total solids, ash, soluble protein and acidity were increased whereas, total protein and pH values were decreased in all treatments like-labneh products. Moreover, the acetaldehyde concentration in like-labneh from all treatments had increased gradually during storage period to reach a maximum after 18 days and its followed by a decreased at the end of storage period. Whereas, the diacetyl concentration had increased till the end of storage period. The organic acids, citric, pyrovic and lactic acids were decreased in like-labneh products after 8 days. Whilst acetic and formic acids were decreased after 4 days in all treatments during storage.

The results from microbiological properties in like-labneh products for all treatments showed that the total counts of bacteria and bifidobacterium lactis had decreased after 18 days and 4 days , respectively, and also, *Streptococcus thermophilus* was decreased and increased after 8 days. Whereas, *Lactobacillus acidophilus* had gradually decreased meanwhile, yeast and moulds were opposite trends.

The sensory evaluation of like-labneh products for all treatments during storage period gave not changes and the best flavour, taste and texture.

It might be concluded that the like-labneh product from incubated barley flour and ABT followed by bifidobacteria and *Lactobacillus acidophilus* gave the best sensory evaluation.

INTRODUCTION

Modern consumers are increasingly interested in their personal health, and expect the foods, they eat to be-beyond and attractive-also safe and healthy. As interest in the link between diet and health gathers pace, many consumers seek ways to feel and stay healthy by eating nutritionally designed foods (Pimiae *et al.*, 2003).

Probiotic foods have steadily gained popularity over the past decades and a wide variety of foods are nowadays used as carriers for probiotic cultures, including fermented milk products (Coeuret *et al.*, 2004), cheese (Stanton *et*

al., 1998), ice cream (Christiansen *et al.*, 1996), fruit juices (Post, 2002), cereal bars (Ouwehand *et al.*, 2004) and infant formulas (Todd, 2003).

Recently interest in the relationship between health and diet has led to increased consumer familiarity with terms such as functional foods and dietary fiber in connection with their potential to reduce the risk of disease, as a consequence, the food industry is faced with the challenge of producing functional foods acceptance to the consumers (Coeuret *et al.*, 2004).

Hull-less barley β -glucan is a major component of soluble dietary fiber. Soluble dietary fibers have become increasingly popular, their consumption being often associated with beneficial effects. In vitro and in vivo studies have demonstrated that certain dietary fibers could reduce the risk of the developing cardiovascular disease because they lead to a decrease in blood cholesterol levels, decreased carbohydrate digestion and hence regulation of postprandial blood glucose and insulin level and and the promotion of a healthy balance of gut microflora (Bhatty,1999).

Within this group of soluble dietary fibers, beta glucan has attracted considerable interest. The use of barley and barley products have also reported in cereal foods such bakery products and pasta and the quality of the end products was evaluated. (Bhatty,1999).

Limited research has focused on the use of barley in dairy products, with several paper reporting the utilization of soya and other legume in the production of a yoghurt like products (Hala *et al.*, 2004). Not many publication results are available with regard to the interaction between barley flour and milk components.

However, little work has been carried out to establish the effect barley on the properties of labneh. Furthermore, this study aimed to investegate the effect of both different types strain, hull-less barley flour and buffaloes' milk on the quality like-labneh product during storage period.

MATERIALS AND METHODS

Materials:

Buffaloes' milk was obtained from plant of Food Technology Research Institute, Agric. Res. Center, Giza, Egypt. Also, butter milk powder was obtained from Agri-Best Australia pty ltd. Hull-less barley seeds was obtained from Field Crops Res. Inst., Agric. Res. Center, Giza, Egypt. Hull-less barley seeds were milled in Laboratory Mill to give a fine powder followed by siving through a screen 25 μ m to separate the flour. Glocono-Delta-Lacton was obtained from Misr milk and Food Company, Cairo, Egypt. Freez-dried culture of (1) yoghurt culture which consists of *Streptococcus salvarious sub thermophilus* Tcc-3 and *Lactobacillus delbrueckii subsp. buligaricus*, (2) *Bifidobacterium lactis* Bb-12, (3) *Lactobacillus acidophilus* N4495 and (4) ABT culture *Lactobacillus acidophilus*, *Bifidobacterium lactis*, and *Streptococcus salvarious sub thermophilus* were purchased from Chr. Hansen's Laboratories, Denmark.

All media used for microbiological analysis were ready made and obtained from Oxoid Co., Hampshire RG 24 OPW- England.

Methods:

Preparation of different stock cultures:

The cultures were prepared by adding few milligram of freeze-dried culture to 100 ml of skim milk contained 10 % total solids and sterilized at 121°C for 2 min. This mixture was incubated at 42°C until the onset of gelation. Two millilitres of culture from this passage were transferred into 100 ml of sterilized skim-milk at 42 °C and, once again, the culture was incubated until a gel had just formed. This second culture was used for the propagation of a stock culture (1 L) for inoculation of the different treatments. Stock cultures were prepared freshly before the production of like-labneh.

Preparation of like-labneh as a concentrate:

Buffalo's milk was heated at 40°C then 5 % butter milk powder and 10 % barley flour were added and blended by using of high speed blender, the total solid in the end mixture was 30 %. The previous mixture was heated at 72°C /10 min and labneh samples were smoothed using a lactic curd homogenizer fitted with different homogenizer heads (D-170 and D-280). Homogenization markedly affected the firmness of labneh and is therefore recommended to smooth the product using homogenizer head D-280 because it causes less turbulence. The mixture was cooled to 40°C and divided into five parts for the following treatments:-

Treatment I: was inoculated by 3% with active stock culture *Streptococcus salivarius subsp thermophilus* TCC-3 and *Lactobacillus bulgaricus* and incubated at 37 °C for 3 h. (control).

Treatment II: was inoculated by 3% with active stock culture *Bifidobacterium lactis Bb-12* and *Lactobacillus acidophilus* N 4495 (1:1) and incubated at 37 °C for 3 h.

Treatment III: was inoculated by 3% with active stock culture ABT and incubated at 37 °C for 3 h.

Treatment IV: was inoculated by 1.5 % with active stock culture *Bifidobacterium lactis Bb-12* and *Lactobacillus acidophilus* N 4495 (1:1) + 0.5 % glucono-delta-lacton and incubated at 37 °C for 3 h.

Treatment V: was inoculated by 1.5 % with active stock culture ABT + 0.5 % glucono- delta-lacton and incubated at 37 °C for 3 h.

The obtained labneh samples were stored at 5 °C in the refrigerator. The chemically, microbiologically and organoleptically were determined in the like-labneh at different storage periods, zero time, 4, 8, 18, and 30 days.

Chemical analysis:

The percentage of titratable acidity, moisture, total nitrogen and soluble nitrogen contents were determined according to the method as described by (AOAC.2000). pH values of all like-labneh samples were recorded by using digital pH meter model SA 720 (Orion, USA). Acetaldehyde and diacetyl contents were determined according to (Lees and Jago, 1969 a, b).

Determination of Organic acid using HPLC apparatus in like-Labneh:

Liquid chromatography (LC): Waters associates equipped with 600E multisolvent delivery system and millennium chromatography workstation was used. Determination was carried out at wavelength 210nm, flow rate 1.5ml/min and ambient temperature Altec column (250×4.6mm). The mobile phase was 0.001% H₂ SO₄ according to Seydim *et al.*, (2000).

Microbiological estimation:

Total bacterial counts, *bifidobacterium*, *lactobacillus acidophilus*, *streptococcus thermophilus* were determined according to the methods described by Houghtby *et al.*, (1992), Lim *et al.*, (1995), Gilliland and Walker (1990) and Ghodusi and Robinson (1996). Whereas, yeast and moulds were determined according to Marshall,(1992). Meanwhile, coliforms was determined as described in the method by Marshall, (1992). Labneh samples were emulsified in 2% (w/v) trisodium citrate, diluted in maximum recovery diluent's (Oxoid) and appropriate were pour plated.

Sensory evaluation:

All resultant Labneh treatments were graded when fresh and at 4, 8, 18, and 30 days of refrigerator storage (5°C) by staff members of Dairy Department, Food Technology Institute, Agricultural Research Center according to (Pappas *et al.* 1996).

RESULTS AND DISCUSSION

Chemical composition of like-labneh product during storage period:

The chemical composition of all treatments like-labneh product using 10 % hull-less barley flour, 5 % butter milk and buffaloes' milk contained 14 % total solids were determined during storage period (30 days). The results are reported in Table (1). The results revealed that the total solids in all treatments had slightly increased till the end of storage period. Whereas, treatments II, III increased from 25.24 and 25.21 % at zero time to 25.94 and 25.83 %, respectively after 30 days. From the results, it noticed that the slightly increased in all treatments like-labneh total solids may be to contain the buffaloes' milk about 14 % total solids and addition of 10 % barley flour. Moreover, ash content had the similar trend as total solids. These results are in accordance with reported by Hassanein *et al.*, (2008).

Titratable acidity and pH values were determined in all treatments like-labneh during storage period. The results showed that the like-labneh treatments contained relatively the highest acidity till the end of storage period. The trend of the changes in pH values of all treatments was opposite to that of acidity which may be led to more lactic acid production as a result of microorganism metabolism (Abd-Allah *et al.*, 1993).

During storage period the total protein decreased in all treatments like-labneh, such as decrease may be due to protein degradation and formation of water-soluble compound. On the other hand the soluble protein on total protein as percent was increased in like-labneh made from barley flour and different strains during storage period. This may be due to the highest present of hull-less flour in soluble protein which caused a higher protein decomposition (Hassanein, 2006).

Acetaldehyde and diacetyl concentration:

The acetaldehyde concentration in labneh samples Fig (1) for all treatments were increased continuously till 18 days storage period and then decreased gradually at 30 days storage period. These results were agreed with the results obtained by (Gorner *et al*, 1972) on the plain yoghurt.

Acetaldehyde can be converted into ethanol by alcohol dehydrogenase (Tamime and Robinson, 1983). This may be explaining the lower amount of acetaldehyde observed during the storage period.

Table (1): Chemical composition of like-labneh product from probiotic fermented barley during the storage period at 5°C:

Treatments	Storage period	T.S	Fat	Ash	T. protein	S.P/T.P	pH	Acidity
	Zero time							
I	Zero time	25.1918	4.0	0.7121	16.399	38.88	5.18	1.13
II		25.2470	4.0	0.8031	16.399	38.88	5.23	1.10
III		25.2085	4.0	0.7401	15.488	35.29	5.41	1.14
IV		25.6018	4.0	1.0139	16.399	33.34	4.95	1.18
V		25.4595	4.0	0.9264	15.488	30.55	5.29	1.19
	4 days							
I	4 days	25.2565	4.0	0.8152	15.829	40.29	4.40	1.15
II		25.3343	4.0	0.9038	15.829	40.28	4.31	1.13
III		25.2852	4.0	0.7706	14.121	38.71	4.48	1.16
IV		25.6088	4.0	1.0209	15.715	35.50	4.46	1.19
V		25.4891	4.0	1.2782	15.146	34.25	4.46	1.23
	8days							
I	8days	25.3448	4.0	0.9182	15.260	41.79	4.37	1.18
II		25.4217	4.0	1.0045	15.260	41.79	4.34	1.16
III		25.3619	4.0	0.8011	12.755	40.64	4.46	1.18
IV		25.6159	4.0	1.1878	15.032	37.88	4.53	1.21
V		25.5188	4.0	1.6318	14.805	34.75	4.06	1.25
	18days							
I	18days	25.4335	4.0	0.7027	14.121	45.16	4.20	1.20
II		25.5964	4.0	0.8375	14.121	45.15	4.35	1.18
III		25.5153	4.0	0.6213	11.312	42.85	4.30	1.19
IV		25.6301	4.0	0.9540	13.666	40.41	4.48	1.22
V		25.5782	4.0	1.3889	14.122	36.50	4.03	1.26
	30 days							
I	30 days	25.5225	4.0	0.6933	11.843	53.84	4.16	1.23
II		25.9459	4.0	0.8118	11.843	53.58	4.21	1.20
III		25.8306	4.0	0.5970	10.011	50.52	4.20	1.20
IV		25.6584	4.0	0.7566	11.633	46.22	4.40	1.24
V		25.6677	4.0	0.8503	12.755	42.16	4.00	1.28

T.S. : Total solids. T.protein : total protein. S.P/T.P: Soluble protein/ Total ptein.

The diacetyl concentration in labneh samples Fig (2) for all treatments were increased gradually during 30 days storage period . These results were in accordance with those reported by Hassanein, (2003).

Organic acids:

The spectrum of organic acids content (g /100g sample) in like-labneh are shown in Figs. from (3 to 7). The pattern of organic acids content in Labneh (.i.e. increased or decreased) was influenced by the metabolic activity of the starter culture. In general the obtained data differs slightly from the level of organic acid content in milk after the fermentation stage .

Acetic and formic acids in Figs (3 and 5) were metabolized after 4 days in all treatments like-labneh products during storage period. Whereas, citric, pyrovic and lactic acids in Figs (4, 6 and 7) had decreased after 8 days in all treatments like-labneh products during storage period. These results are in agreement with Marsili *et al.*,(1981).

figs

Microbiological properties:

The results in Table (2) showed that the changes in total count of different treatments of like-labneh products during storage period at refrigerator temperature 5 °C. The results indicated that gradual increasing was observed throughout the storage period to reach a maximum after 18 days, in all treatments and then decreased to the end of storage period. The results indicated that the total count were higher in treatment II and III Labneh like product from barley.

Table(2): Microbiological analysis (log cfu/g) in like-labneh product from probiotic fermented barley during the storage period at 5°C:

Treatments	Storage period	T.C.	Bif.	Lb.acid.	S.ther.	Y. & M.	Coliform
	Zero time						
I	Zero time	7.42	N.D	N.D	8.44	0	N.D
II		7.65	7.59	9.2	N.D	0	N.D
III		7.78	7.72	8.37	8.64	0	N.D
IV		7.29	7.34	6.95	N.D	0	N.D
V		7.22	6.68	8.34	6.59	0	N.D
	4 days						
I	4 days	7.87	N.D	N.D	8.33	0	N.D
II		8.87	8.17	8.37	N.D	0	N.D
III		8.34	8.11	7.53	8.01	0	N.D
IV		8.12	7.54	6.13	N.D	0	N.D
V		8.12	6.95	6.51	6.21	0	N.D
	8days						
I	8days	7.95	N.D	N.D	7.99	2.0	N.D
II		8.98	6.67	7.67	N.D	2.97	N.D
III		9.29	6.59	6.91	7.99	2.3	N.D
IV		8.33	6.12	5.95	N.D	2.7	N.D
V		8.39	5.55	5.77	5.99	3.1	N.D
	18days						
I	18days	8.38	N.D	N.D	8.11	3.4	N.D
II		9.20	5.7	6.31	N.D	4.1	N.D
III		9.57	5.5	5.80	7.88	3.91	N.D
IV		8.65	5.11	4.98	N.D	4.5	N.D
V		8.59	5.19	4.81	6.11	4.13	N.D
	30 days						
I	30 days	7.84	N.D	N.D	8.09	6.3	N.D
II		8.42	5.1	5.44	N.D	5.9	N.D
III		8.54	4.95	5.18	8.60	5.63	N.D
IV		7.31	4.39	4.15	N.D	6.3	N.D
V		8.26	4.29	4.07	6.08	5.4	N.D

T.C. : total bacterial counts.

Bif. : Bifidobactrium lactis.

Lb. acid. : Lactobacillus acidophilus.

S. Ther. : Streptococcus thermophilus.

T. & M. : Yeast and mold.

It might be due to the presence of probiotic bacteria and barley flour in this product which reflecting of total count till 18 days. Activity of lactic acid bacteria in barley flour fermented had increased the acidity and consequently decrease the total bacteria count. Mehanna *et al.*,(2002).

The viability of *Bifidobacterium lactis* Bb-12 in labneh like product with barley flour during storage period at 5 °C was presented in Table (2). The log count of *Bifidobacterium lactis* Bb-12 in treatment II, III, IV and V were 7.59, 7.72, 7.34 and 6.68 cfu at zero time and decreased to 5.1, 4.95, 4.39 and 4.29 cfu respectively, at the end of storage period. The decrease in the viable of *Bifidobacterium* count in all treatments may be due to the effect of raise of acidity, which performed mainly from lactic and acetic acid which led to decline in the viable *Bifidobacterium* after 20 days of the storage period (Gomes *et al.*, 1995). Results are in agreement with (Chou and Hou, 2000, and Laine *et al.*, 2003), who reported that the *Bifidobacterium* can grow and reduce the pH of Oat-based medium and soy milk were the viable decrease in the storage.

The results in the same Table indicated that the log count of *Lactobacillus acidophilus* in treatment II, was higher than other treatments at zero time III, IV and V. On the other hand, the count of *Lactobacillus acidophilus* was higher treatments IV and V. It may be due to the mixed strains of *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Bifidobacterium* in treatment III. The results confirmed by (Anderson and Gilliland, 1999) who reported that *Lactobacillus acidophilus* strains live in excellent symbiotic with *Bifidobacterium* which provide the necessary growth stimulant to the former.

The same Table showed the viability of *Streptococcus thermophilus* in like-labneh product with barley flour during storage period at 5 °C. The count of *Str. thermophilus* in treatment I, II and V were 8.44, 8.64 and 6.59 log cfu/g respectively at zero time, gradually decreased to 8.09, 8.6 and 6.8 log cfu / g respectively after 30 days the end of storage period. These results are agreement with the results that obtained by (Vinderola *et al.*, 2000) who reported that there are not different between the initial count of *Streptococcus Thermophilus* in Argentinean yoghurt in fresh yoghurt and at the end of storage.

Data in the same Table illustrate the yeast and mould counts of like-labneh products during storage at 5 °C. Generally, yeasts and moulds could not be detected in zero time and 4 days during storage. Moreover, yeast and mould counts could be detected and counted at 8 days in all treatments and slightly increased during storage. There were no differences between most treatments.

Yeast and mould counts slightly increased in the samples this may be due to the contamination from polyethylene. These results are in line with the results reported by (Mehanna *et al.*, 2002), they found that, the yeast and mould began to appear after 7days of storage and (Al-Kadamany *et al.*, 2003), who found that the yeast and mould began to appear after 20 days of storage period.

All samples of like-labneh products fresh and stored made with different treatment were coliform bacteria free. This may due to the efficient heat treatment and high sanitation conditions during manufacture and storage. These results are in agreement with the results reported by (Gafour, 2005) who reported that the coliforms were not detected all over storage period in Ultrafiltration like cheese at the beginning and end of the storage periods.

Sensory evaluation:

Table (3) shows the effect of all the previous treatments on the organoleptic properties of the resultant labneh during 30 days of storage. It could be concluded that the like-labneh gave the best flavour, taste and texture among the all treatments of like-labneh.

Table(3): Organoleptic propertise of like-labneh product from probiotic fermented barley during the storage period at 5°C:

Tretments	Storage period	Flavour (50)	Body& Texture (40)	Appearance (10)	Total (' 100)
I	Fresh	43	35	9	87
	4 Days	43	35	9	87
	8 Days	43	36	9	88
	18 Days	44	37	9	90
	30 Days	44	37	9	90
II	Fresh	42	35	9	86
	4 Days	42	35	9	86
	8 Days	43	35	9	87
	18 Days	44	36	9	89
	30 Days	45	36	9	90
III	Fresh	44	35	9	88
	4 Days	44	35	9	88
	8 Days	45	36	9	90
	18 Days	46	37	9	92
	30 Days	47	37	9	93
IV	Fresh	41	34	9	84
	4 Days	41	35	9	85
	8 Days	42	35	9	86
	18 Days	43	36	9	88
	30 Days	44	36	9	89
V	Fresh	42	35	9	86
	4 Days	42	35	9	86
	8 Days	43	35	9	87
	18 Days	43	36	9	88
	30 Days	43	36	9	88

Generally a like-labneh product from plant foodstuffs (barley flour) and buffaloes milk has been developed. It had been viability of lactic acid bacteria and probiotic bacteria during storage period 30 days. The lactic acid bacteria were very important to get good acceptability of fermented products and concentrated yoghurt (like-labneh product) aroma.

The mixed substrate could be considered raw material for production of fermented foods, or as extender for yoghurt and imitation dairy products.

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تأثير السلالات البكتيرية المختلفة علي إنتاج شبيه اللبنه من اللبن الجاموسي و الشعير العاري

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يحضر منتج شبيه اللبنه بواسطة اللبن الجاموسي المحتوي علي 14 % مواد صلبه كلية مع 10 % دقيق شعير و 6% لبن خض مجفف . وهذا المخلوط تم تحضيره مع سلالات بكتيرية ليعطي 5 معاملات . المعاملة الأولى تحتوي علي 3% بادئ الزبدي والمعاملة الثانية تحتوي علي 1.5% بكتيريا البيفيدوبكتيريا و 1.5% لاكتوباسلس اسيدوفيلس و المعاملة الثالثة تحتوي علي 3 % بادئ مختلط ABT أما المعاملة الرابعة فتحتوي علي بادئ المعاملة الثانية بنسبة 1.5 % بالإضافة إلي 0.5 % جلوكون دلتا لاكتون والمعاملة الخامسة تحتوي علي بادئ المعاملة الثالثة بنسبة 1.5 % مع 0.5 % جلوكون دلتا لاكتون . الاختبارات الكيماوية و الميكروبيولوجية والحسية تم تقديرها في منتج شبيه اللبنه علي فترات تخزين مختلفة من الطازج ، 4 ، 8 ، 18 ، 30 يوم علي درجة حرارة 5 ° م .

أوضحت النتائج أن الجوامد الكلية والرماد و البروتين الذائب منسوباً الي البروتين الكلي والحموضة حدث لها زيادة في البروتين الكلي و قيم ال pH حدث لها انخفاض في كل معاملات المنتج . أما بالنسبة الي الأسيتالدهيد فقد حدث له زيادة تدريجية أثناء التخزين ليصل إلي أقصى زيادة بعد 18 يوم ثم إنخفاض حتي نهاية فترة التخزين في جميع المعاملات ... بينما الداي استيل حدث له زيادة في جميع المعاملات وحتى نهاية فترة التخزين. الأحماض العضوية (الستريك - البيروفيك - اللاكتيك) حدث لها انخفاض في المنتج بعد اليوم الثامن بينما (الأستيك - الفورميك) انخفضت بعد أربعة أيام في كل المعاملات .

أوضحت النتائج أن الخواص الميكروبيولوجية في منتج شبيه اللبنه لجميع المعاملات حدث لها انخفاض بعد 18 يوم بالنسبة للعدد الكلي البكتيري وأربعة أيام بالنسبة للبيفيدوبكتيريا . أما بالنسبة للثيرموفيلس فقد حدث لها انخفاض حتي ثماني أيام ثم زيادة بعد ذلك . بينما الأسيدوفيلس انخفضت انخفاضاً تدريجياً حتي نهاية فترة التخزين أما الفطر والخميرة فكانت نتائجها عكس نتائج الأسيدوفيلس.

التقييم الحسي لمنتج شبيه اللبنه بالنسبة لجميع المعاملات أثناء فترة التخزين ، أوضحت النتائج عدم وجود أي تغيير يذكر بالمقارنة بالعينة القياسية. ومن هنا يمكن أن نوصي باستخدام سلالاتي خليط ABT وكذلك البيفيدوبكتيريا مع الأسيدوفيلس لتصنيع شبيه منتج اللبنه المدعم بالشعير العاري.

