ASPECTS IN PRODUCTION AND EVALUATION OF PROBIOTIC KISHK

Neimat A. H. Elewa and Wedad A. Metry

Dairy Department, Faculty of Agriculture, Fayoum University, Egypt.

Abstract: The Egyptian traditional kishk as functional food could be developed to probiotic food. So *Lactobacillus acidophilus* was used as probiotic bacteria in place of natural lactic acid bacteria (LAB) beside the using of soybean or burghul as cereal source and acidophilic whey, soy milk or skim milk in place of fermented milk to produce probiotic Kishk as follows: 1- Burghul and laban El- Zeer as native kishk (control), 2- Burghul with acidophilic milk, 3-Soybean dough (soybean flour with 2.5% sodium chloride solution at ratio 1 : 4) with acidophilic milk, 4- Burghul with acidophilic soybean milk and 5- Soybean dough with acidophilic whey. Kishk samples were stored for 3 months at room temperature for microbiological examination (viable counts of *L. acidophilus*, spore former , yeast, mould and total viable counts) and chemical analysis (total solids, titratable acidity, pH, ether extract, total nitrogen , water soluble nitrogen , crude fibers, ash, sugars and minerals contents) which also done at 37^{o} C. The results revealed that total solids, titratable acidity, ether extract, total nitrogen, water soluble nitrogen, crude fibers and ash contents increased during storage .On the other hand, crude fiber in burghul based Kishk was higher than that in soy based Kishk. In contrast, the ash , total nitrogen , total sugars and pH in soybean based Kishk were higher than that in burghul Kishk. As well as Fe, Zn, Mg and Ca recorded high content in soy Kishk. Using of acidophilic milk and burghul reflected in highest rate of maintenance viable *L. acidophilus*. While the lowest one with using soybean flour and whey. Spore former , yeast and mould counts were present in averaged from log 3.9 to 5.34 and 3.1 to 3.9 cfu/g respectively in different treatments.

Keywords: *L. acidophilus*, soybean, soymilk, burghul, Kishk, chemical and microbiological analysis.

Introduction

Careful consideration is given to the safety of functional foods, the key to an adequate assessment of the health related benefits is the availability of accepted sensitive and reliable biological markers. A recent trend to ensure such functionality is to incorporate probiotic bacteria into fermented foods. One recent incorporation of such probiotic bacteria as *L. acidophilus* in fermented dairy products, addition of these beneficial cultures imparts a net work of health attributes, which encompass improved nutritional value, reduction of lactose intolerance, antagonistic action towards enteric pathogens, anti carcinogenic effects and immune modulation. This communication focuses on emerging evidence of the protective role ascribed to fermented dairy products. The growth stimulation and acidification of probiotic bacteria as *L. acidophilus* in colon, over undesirable or pathogenic microorganisms, hence increasing their viable all numbers, as well as production of short chain fatty acids (SCFA) these acids may play a functional role, since they provide metabolic energy for the host and acidification of the bowel (Sghir *et al.,* 1998). Recall also that milk and dairy products contain, in their native forms, an array of components that provide critical elements for nutritional fulfillment and immunological protection, as well as biologically active substances to both neonates and adults. More recently prebiotics have been widely added to various functional dairy products. However, using of wheat which contain starches, dietary fibers, other non absorbable sugars, inulin and oligosaccharide (Malcata *et al.,* 2005) play as prebiotic agent in symbiotic Kishk . Lactic acid fermented milks include Malaysian tairu(soybean milk) (Steinkraus, 2002). Indonesian Tempeh, Natto and Hamanatto in Japan were as traditional fermented soybean (Van Veen and Steinkraus, 1970). Furthermore, the consumption of fermented soybean foods has increased in recent years and seems destined to continue increasing in the future, especially in light of significant cost advantages in producing food proteins of plant versus animal origin(Beuchat, 1984). Many researcher indicated that , soybeans contain a number of anti nutritional factors that reduce their nutritional value and must be eliminated before the beans can be used in preparing kishk. The heat treatment in alkaline medium has been reported to be effective in destroying trypsin inhibitor and other anti nutritional factors in soybean(Luttrell *et al.,* 1981).

Interactions among components in functional foods, availability of nutrients and effects of processing upon the health-enhancing potential of functional foods. Therefore, the use of probiotic bacteria in the manufacture of kishk or health related products, alone or together with prebiotics - synbiotic products that have synergistically beneficial effect upon the host by improving the survival and/or implantation of selected live microbial strains in gastrointestinal tract may contribute to general health.

Finally, the aim of this investigation represented in the production of probiotic kishk through out the using of probiotic bacteria as *L. acidophilus* and prebiotic matters as wheat or soybean beside of whey & milk or soymilk. Some chemical and microbiological analysis were done to evaluate the net work

Materials and Methods

Materials:

Fresh milk was skimmed using Alpha - laval cream separator and skimmed milk was used to prepare acidophilus milk. *L. acidophilus* strain was obtained from Chr. Hansen's laboratory; it was activated by propagated in a sterile skim milk by several transfers before use. Soybean milk and soybean flour were obtained from INTSOY / Food Science Soymilk process, Food Technology Research Institute, Agriculture Research Center, Giza, Egypt. Sweet whey produced as by-product from Ras cheese making. Burghul (El-Doha) and commercial edible grade sodium chloride were bought from a local market.

Methods:

1-Preparation of soaked burghul

Burghul was soaked into 2.5 % sodium chloride solution at ratio 1: 2.5 (w/v) for 2 hours.

2-Preparation of soybean dough

Soybean flour was wetted with 2.5 % sodium chloride solution in proportion 1: 4 (w/v) to get soybean dough.

3-Preparation of acidophilic milk, acidophilic soybean milk and acidophilic whey

Starter culture of *L. acidophilus* was inoculated in fresh skim milk, soybean milk or whey at 3 % level and incubated at 37 ^{o} C for 13 – 15 h to ferment then keep at 5 ± 1 ^{o}C.

Experimental procedures 4-

Five treatments of modified kishk were prepared as follows:

1-Native kishk was prepared traditionally in house farmer in Fayoum governorate from burghul and laban El-zeer(2:1) depending on natural lactic acid bacteria (LAB) fermentation as Control (C).

2- Soaked burghul was mixed with fresh acidophilic milk in 2:1 proportion (B A).

3- Soybean dough was mixed with acidophilic milk in ratio 2:1 (SA).

4- Soaked burghul mixed with acidophilic soy milk in ratio 2: 1 (BS).

5- Soy bean dough was mixed with acidophilic whey in ratio 2 : 1 (SW).

The resultant Kishk samples were stored for 3 months at room temperature (20 ± 2 ^{o} C) and 37 ^{o} C for chemical analysis, but microbiological analysis was carried out at 20 ± 2 ^{o} C. Kishk samples were analyzed at intervals 3 months.

5- Methods of analysis

5.1. Microbiological analysis.

Total viable counts (TVC) and spore former counts were determined by using plate count skim milk agar according to Houghtby *et al.*(1992) .

Viable counts of *Lactobacilli* were determined using Rogosa agar (Lactobacillus selective agar) and coliform counts using MacConkey broth (purple) according to Merck (1988). The yeast and mould counts were enumerated according to Marshall (1992) using potatoes dextrose agar.

5.2. Chemical analysis:

Kishk samples were tested for total solids, titratable acidity, ether extract, total nitrogen, water soluble nitrogen, crude fibers and ash contents as mentioned in A. O. A. C (2000). Reducing and total sugars were determined as described by Dubois *et al.*(1956). The pH values were measured using a pH meter, model Kent EIL 7020, U.K .Measurement of browning (absorbance at 420 nm) was carried out as described by Ranjanna (1977). Iorn, zinc, lead and magnesium were determined in Kishk ash according to A.O.A.C (2000) using atomic absorption spectrophotometer (ZEISS, AAS 5 , Germany), while calcium and magnesium were determined by versenate (EDTA) titration method using Eriochrome black–T as an indicator and calcium was then calculated by the difference between calcium plus magnesium and magnesium.

Results and Discussion

Microbiological analysis:

L. acidophilus viable counts

An over look on Table (1) indicated that *L. acidophilus* was in gradual decrease during storage period. The differentiation of raw matter from kishk was made reflected on the rate of decrease which consequently affected on the continuation of viable cells. However, in this research, it was very important to maintain the probiotic bacteria (*L. acidophilus*) life for more long time. Furthermore, BA treatment registered the highest proportion of viable *L. acidophilus* cells after 100 days of storage, it represented 0.89% followed by SA and BS which were 0.81 and 0.77%, respectively, may be that confirmed the importance of acidophilic milk and burghul presentation, while the lowest rate of maintenance was observed in SW which represented 0.28% with regards to control sample, it recorded the lowest number of Lactobacilli viable cells (0.008%) as detected in the same table. However, lactic acid bacteria have grown well in soymilk, but they produce less acid than they do in dairy milk that is mainly because soymilk lacks in mono saccharides and the disaccharide (lactose). Instead, it contains sugars such as sucrose, raffinose and stachyose, which are not readily digestible by many lactic acid bacteria due to their lack of galactosidase (Liu, 1997).

Total viable counts (TVC)

Data represented in Table (1) indicated that total viable counts in all kishk treatments and control were in gradual decrease in around 3 and 4 log cfu/ gm, respectively. It could be noticed that TVC in soy- based kishk were less than that in burghul- based

kishk till the end of storage period. The results confirms the findings of Park *et al.* (1993) and Abu-Zaid (2000) who reported that soy flour was microbiologically safe during prolonged storage at 37 ^{0}C. Also the same author found that soy milk is considered a suitable growing medium for pathogens which a superior to the normal milk which emphasize the role of soy milk products as carriers in disease transmitter. It is found that, soy sauce had an antimicrobial activity arised from a combination between some compounds released from the fermentation process. This antimicrobial compounds prevent spoilage and/or pathogenic microorganisms (Abu-Zaid, 2005). On the other hand, El-Gendy (2001) reported that the total count of kishk samples varied widly from 2.900 to 1.000.000 organism per gm of dry weight. Any way the reduction of total count attributed with the presence of *L. acidophilus* which led to reduction in pH corresponding to raise in developed acidity. Also, *L. acidophilus* antagonisms prevent or inhibit the growth of many organisms as recorded by Alm(1983), Elewa (1992) and El-Gendy (2004). In addition of the non hygroscopic nature of kishk led to more reduction in microorganisms count. Coliform, Sporeform , Mould and yeast counts.

The data recorded in Table(2) revealed that coliforms were presented in some treatments as SA&BS in a few numbers 2.10 and 2.98 log cfu/gm respectively. Coliforms organisms may be implicated in food illness among the consumers. The additives were the most important source of contamination with coliforms. In general, It may be used as indicator for processing sanitation. However according to American Public Health Association (APHA, 1994) guidelines, none of enteropathogenic *Escherichia coli, Vibrio parahemolytic, Listeria monocytogenes, Camylobacter jejuni* should be present. Also, no Yersinia enterocolitica and the final Standard Plate Count (SPC) should not exceed 20.000 cfu/g. Furthermore, coliforms should be absent in 1.0ml samples of soy milk products. With regards to aerobic spore formers in kishk treatments, they varied from log 3.9 to 5.34 cfu/gm. The higher occurrence of aerobic spore forms may be due to their heat resistance. They produce heat resistant proteolytic and lipolytic enzymes which may cause spoilage in the products (Varnam& Sutherland, 1994 and Armesto& Alastair, 1997).

The data given in Table (2) illustrated that yeasts and moulds were detected in the treatments in log 3 cfu/gm while not detected in BA. The presence of yeast may be due to their frequent presence in cereal as well as their high resistances of heat treatment (Gourama& Bullerman, 1995, Ropert, 1997 and Li& Li, 1998). As moulds are widely distributed as environmental contaminants of air, water and soil, they are responsible for spoilage of kishk. Finally, acidophilic kishk as probiotic food is resistant towards development of food spoilage or pathogenic microorganisms due to *L. acidophilus* antagonisms, their relatively low pH(after reconstitution), Its none hygroscopic nature ,so that it can be stored in open jars for 2-3years without obvious deterioration.

Table(2): Counts of coliforms, sporeforms , yeasts and moulds counts in probiotic Kishk during storage at room temperature .

Treatments*	Coliform counts (log cfu/gm)	Sporeform counts (log cfu/gm)	Yeast & mould counts (log cfu/gm)
control	ND	4.11	3.20
BA	ND	5.34	ND
SA	2.10	3.90	3.90
BS	2.98	5.10	3.50
SW	ND	4.54	3.10

ND: Not detected

Chemical analysis:

Total Solids % (TS), Ash and Crude fibers (%)

As observed from Table (3) TS averaged from 90.02 to 92.19 % in fresh treatments. However, gradual increase of TS was detected in all treatments during storage. The rate of increase averaged from 1.0 to 1.9% when stored at room temperature (20 ± 2^0 C), also it was little bit low in the TS with the storage at 37^0 C. Any way Kishk recognized as non hygroscopic matter, that explain why total solids increased during storage. Furthermore, these results were in agreement with Attia and Khattab (1985), and Tamime *et al*.(1997).

Data in the same Table indicated that ash content in fresh burghul-based kishk recorded 5.02 and 6.08 % in control and BA whilst it was 6.65 and 8.09 in SA and SW whereas soybean dough was added. However, BS registered the highest ratio of ash whereas was 10.19% that attributed with the used of soy milk. Storage progress reflected in slightly increase in ash at room temperature or even at 37^0 C. These results were in agreement with Morcos *et al.* (1973)& Attia and Khattab (1985).

As also, seen in Table (3) crude fibers in burghul- based kishk was higher than that in soy-based kishk in all treatments. They represented 2.25, 2.47 and 2.30% in fresh control, BA and BS, respectively corresponding to

1.03 and 1.11% in SA and SW, respectively. That was almost in agreement with Demerdash (1960) and Tamime *et al.* (1997). However, slightly increase was observed in all treatments with the progress of storage period (120 days). The rate of increase was around 0.04% in both of treatments storage at room temperature or at 37 ^{0}C. As detected cereal was the responsible one for the fiber ratio.

Ether extract contents% (EE)

Data in Table (4) recorded EE content in kishk treatments, the percentage of EE depends on the used cereal. However, using of burghul in control, BA and BS registered 0.16, 0.14 and 0.16 % EE respectively, whilest the substitution of burghul with soybean dough doubled the EE content to 0.35 and 0.32 in SA and SW respectively. **Brisha *et al.* (2002)** reported that soybean & soymilk contained 30 – 35 % and 2 – 4 % fat on dry matter, respectively. Furthermore, slightly increase was noticed in EE content in all treatments during storage at room temperature or even at 37^{o} C this may be due to the little loss of moisture content in kishk which recognized as non hydroscopic matter , also , it could be signed to that the rate of EE increase in treatments stored at 37^{o} C was more than that storaged at room temperature may be that attributed with the less loss of moisture content in the former ones as indicated in Table (3) .

Total Nitrogen content% (TN)

Data presented in Table (4) indicated that TN in soy bean Kishk treatments (SA and SW) was higher than that in burghul kishk treatments (control and BA) whilst BS which made of burghul and soy milk had TN content less than soy bean Kishk. However, TN represented 5.19 to 5.37% in soy bean kishk treatments corresponding to 2.23 to 2.56 % in burghul kishk. However, burghul soymilk kishk (BS) relatively registered the lowest TN. Brisha *et al.* (2002) reported that soybean and soymilk contained 50-55% and 10-13% protein (on dry weight), while burghul kishk contained 23.5% protein (El-Gendy, 1983 and Tamime *et al* .,1997). That may be explained the results. Furthermore, progress of slight increase in TN was detected in all treatments during storage at room temperature or at 37 ^{0}C , but the rate of increase for the former was less more than the latter may be that in compatibility with the loss of moisture content as detected in Table(3).

Water Soluble Nitrogen% (WSN)

Results in Table(4) registered that the highest WSN was in BA and SW which represented around 0.6% may be that due to the relative high viable *L. acidophilus* count in BA as indicated in Table(1) which characterized by proteolysis activity as mentioned by Sneath, *et al.* (1986) and also confirmed by the relative high acidity developed in this treatment as indicated in Table (6).

Also with regards to SW, may be the relative high content of WSN due to the whey protein which represented 0.78 % compared to 0.70 % in milk as reported by Metry (1995) . SA and BS treatments which made from soybean dough and soymilk, respectively, had low WSN whereas around 0.3%, they still above the control that had the lowest WSN content (0.17%).The rate of increase was around 0.1% with the storage at 37 ^{0}C while it was more than that at room temperature in all treatments except in control, it was 0.05%. The obtained results indicated that, the rate of accumulation of WSN increased as the storage period increased in all Kishk treatments. This was attributed by the rate of proteolysis throughout the storage period (Daigle, *et al.*, 1999 and Osman & Abbas, 2001).

Sugars contents (%)

As seen in Table (5) reducing sugars present in small quantities in all kishk treatments. However soy–based kishk SA and SW had higher reducing sugar content than other treatments it represented around 0.74 and 0.70 %, respectively, corresponding to around 0.46 % as detected in control , BA and BS (burghul kishk). During storage little increase in reducing sugars was detected in all treatments. The rate of increase with storage at room temperature was partially lower than that at 37^{o}C. However, El–Gendy (1983) reported that although most of the reducing sugars and microbial flora of the wheat were removed by boiling and during the drying of the cooked grains (belila) a very dense population of diastase – producing bacteria mainly *B. subtilis*–developed and markedly enriches the reducing sugar content of the substrate.

Data presented in Table (5) recorded that total sugars were relatively higher in soy–based kishk than that in burghul kishk. Whilst the highest value was registered 19.34 % in BS treatment which made of burghul and soymilk. Brisha (2002) reported that soymilk and soybean contained 80–84 and 3–5 % carbohydrate (on dry matter), respectively , may be that explain the obtained high value with soymilk kishk treatment. However, soybean kishk SA and SW registered 14.52 and 14.15%, corresponding to 13.2 and 10.23% in BA and control respectively. Furthermore, slight decrease in total sugars was observed in all treatments during storage, the rate of decrease when storage at room temperature was less more than that at 37^{o}C. This decrease may be due to the consumption of sugar by microorganisms' metabolism or in browning reactions. Non reducing sugars as detected in Table (5) recognized as the result of subtraction of total sugars and reducing sugars. Non reducing sugars were less active than reducing sugar either in chemical reactions or in microbiological metabolism. Mital (1974) found that, soybean milk serves as a suitable medium for propagation of lactic acid bacteria. However, *L. acidophilus*

utilized only glucose and sucrose while *L. bulgaricus* utilized only glucose.

Titratable acidity

Data represented in Table(6) showed development of titratable acidity in all kishk treatments as a result of metabolic activity of *L. acidophilus* and persistant metabolic activity of other lactic acid bacteria during storage (Berrocal *et al*., 2005). Acid resistance and acid tolerance bacteria are important virulence determinants that contribute to the survival and pathogen city of infectious food borne pathogens to cause disease. Acid resistance increases the portion of the population that survives gastric infectivity once the pathogens attaches to the intestinal tract (Peterson *et al*., 1989, Jayaro and Yung, 1999).)

pH Values

Evaluation of pH values as detected in Table(6) confirmed that pH values in soy- based kishk BS, SA and SW were slightly higher than that in burghul- based kishk. However, the lowest pH value registered 3.9 in BA, corresponding to 5.00 in SA. Also, pH values were in gradual decrease during storage, that particularly attributed with the viable *L. acidophilus*. However, addition of whey powder, glucose, or lactose to soymilk enhanced acid production by some lactic acid bacteria as well as *L. acidophilus*, it lowered the pH of soymilk indicating its ability to use soymilk carbohydrates for acid production (Abu - Zaid 2000). Finally, it could be concluded that both of burghul and soy- based kishk contained viable *L. acidophilus* as probiotic bacteria and noticeable acidity till 120 days of storage. Burghul based kishk was higher than soy kishk in these parameters during 120 days. Furthermore, the relatively low pH (after reconstitution of water) makes kishk unattractive to pathogens and food spoilage organisms (Van Veen and Steinkrous, 1970). They also added the final product is not hygroscopic and can be stored in open jars for 2-3 years without obvious deterioration.

Color development

Results in table (6) illustrated the changes in the absorbance at 420 nm of kishk treatments which were taken as indicator of browning reactions and darkness of color in kishk . The deepest brown was observed in the treatments made of soybean flour SA and SW then that contained soymilk BS whilst the lowest ones were control , BA and BS which contained burghul , may be that due to the high amino acids content in soybean which react with reducing sugars in non enzymatic browning reactions resulting in brown color development .Gradual increase color darkness was continued during storage period in all treatments by the same ratio whereas were 0.588 , 0.795 and 3.66 in SA , SW and BS, corresponding to 0.218 & 0.324 in control and BA respectively. Also, it could be observed that the

soybean whey kishk registered the highest absorbance value due to the relative high sugar content in whey and as well as the high amino acids content in soybean. Furthermore, the raising of storage temperature to 37^{o}C reflecting in more brown color in all treatments that attributed with the speed reaction increase vs. temperature increase according to Elewa (1982).

Mineral contents (ppm)

An overlook in Table (7) revealed that although soybean based Kishk had higher content of Fe , Zn , Mg and Ca than burghul based Kishk, the presence of soymilk took values less than that in soybean treatments. On the other hand, Pb content was averaged between 3.0 to 4.9 ppm in all Kishk treatments. Tamime *et al.* (1997) indicated that appreciable quantities of Fe , Cu and Mn were found in Kishk paralleling the mineral composition of the cereal of which it was made . However Toufeili *et al.* (1998) suggested that substituting whole wheat meal for burghul in the formulation of Kishk enhances the availability of Ca , Fe , Mg and Zn without undue effects on the acceptability of the final product.

Table (7): Mineral content (ppm) of probiotic Kishk.

Treatments*	Fe	Zn	Pb	Mg	Ca
C	29.13	7.08	3.02	51.02	91.08
BA	22.24	3.78	4.23	45.21	109.46
SA	61.03	35.43	4.97	112.72	445.40
BS	25.76	24.50	4.43	46.27	91.12
SW	57.04	36.24	3.88	78.81	316.28

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بعض الاتجاهات في أنتاج وتقييم الكشك الحيوى

نعمت على حسن عليو ، وداد عزب مترى

قسم الألبان كلية الزراعة جامعة الفيوم - مصر

يمكن تطوير الكشك المصرى النقليدى من غذاء وظيفى الي غذاء حيوى (دعامى probiotic) . لذا أستخدم *Lactobacillus acidophilus* كبكتريا دعامية لتحل محل بكتيريا اللاكتيك الطبيعية هذا إلى جانب أستخدام فول الصويا أو البرغل كمصدر للحبوب. كما أستخدم الشرش الاسيدوفيلى ولبن فول الصويا وكذلك اللبن الفرز كبديل للبن المتخمر وذلك لأنتاج كشك حيوى دعامى كالاتى :

1-كونترول من الكشك التقليدى (برغل مع لبن الزير).

2-برغل مع اللبن الاسيدوفيلى.

3-عجينة من دقيق فول صويا (ومحلول ملحى2.5 % بنسبة 4:1) مع اللبن الاسيدوفيلى .

4-برغل مع لبن فول الصويا الاسيدوفيلى.

5-عجينة فول صويا مع شرش أسيدوفيلى.

تم تخزين عينات الكشك لمدة 3 شهور على درجة حرارة الغرفة 2 ±20° م وتم تحليلها ميكروبيولوجيا(الاعداد الحية من *Lactobacillus acidophilus* والإعداد الحية الكلية و الجراثيم و الفطريات و الخمائر). كذلك تم تحليلها كيميائيا على نفس الدرجة و أيضا على 37م لتقدير المواد الصلبة الكلية والحموضة المعايرة و المستخلص الاثيري و النيتروجين الكلى و النيتروجين الذائب فى الماء و الالياف و الرماد.

هذا و قد أظهرت النتائج عن زيادة فى المواد الصلبة الكلية و الحموضة المعايرة و المستخلص الاثيرى و الالياف و محتوى الرماد أثناء التخزين. من جانب اخر تعتبر الالياف فى كشك البرغل أعلى منها فى كشك فول الصويا. وعلى العكس من ذلك النيتروجين الكلى و السكريات الكلية و كذلك الـ pH حيث كانت أعلى فى كشك فول الصويا عنها فى كشك البرغل. و بالمثل كان الحديد و الزنك و المغنيسيوم والكالسيوم حيث سجلت أرتفاعا فى كشك فول الصويا المستخدم معه اللبن الاسيدوفيلى. كذلك سجل كشك البرغل أرتفاع فى معدل بقاء خلايا *Lactobacillus acidophilus* بينما كان أقل معدل مع كشك فول الصويا و الشرش. كذلك تواجدت الجراثيم والخمائر و الفطريات بنسب تراوحت بين لوغاريتم 3.9 – 5.34 ، 3.1 – 3.9 CFU/g على التوالى فى كل المعاملات.