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# Enhancing the Vitality of Free Fat UF-Soft Cheese Supplemented with Some Nutritious Additives by Adding Probiotic Bacteria

Nesreen M. Nasr<sup>1\*</sup>; Hassan M. H. Hassaan<sup>2</sup>; Essam A. S. Khalifa<sup>2</sup> and Wedad A. Metry<sup>1</sup>

<sup>1</sup> Dairy Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt.

<sup>2</sup> Dairy Technology Research Department, Animal Production Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.

#### ABSTRACT

Probiotics are live microorganisms, which provide health benefits when they are presented in adequate amounts. A dairy product such as cheese is a suitable carrier for the probiotics into the human intestine. Therefore, this study aimed to use probiotic bacteria to improve the vitality and quality of free fat ultrafiltrated (UF) white soft cheese supplemented with 5% fresh mushroom (FM) or 6% bottle gourd seeds powder (BGSP). Two different starters (Lactobacillus acidophilus or Bifidobacterium lactis) were used individually. Free fat buffalo's milk retentate was divided into 7 portions: the first portion (without probiotics or additives) was considered as a control, while the next three portions were inoculated with Lb. acidophilus LA5 and no additives, 5% FM and 6% BGSP ( $L_1$ ,  $L_2$  and  $L_3$ , respectively). The last three portions ( $B_1$ ,  $B_2$  and  $B_3$ ) were inoculated with Bif. lactis and supplemented like previous group. Samples of all resultant cheese were stored at 5±1 °C for 28 days and analyzed for chemical, microbiological and sensory attributes. The results showed that moisture contents of fresh probiotic treatments supplemented with FM were higher than other treatments while, total nitrogen, water soluble nitrogen/total nitrogen, fat and fat/dry matter were higher in cheese supplemented with BGSP at the end of storage. The highest probiotic viability was recorded by Bifidocheese supplemented with BGSP (B<sub>3</sub>) at 14 days of storage. Both, Acidophilus and Bifido-free fat soft cheese supplemented with 5% FM had the best sensory properties. Therefore, it is recommended to produce Acidophilus and Bifido-free fat UF-soft cheese supplemented with 5% FM or 6% BGSP as a new vital product with healthy properties.

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**Keywords:** Probiotic, *Lb. acidophilus, Bif. lactis*, free fat, White soft cheese, Bottle gourd seeds powder, Mushroom, Ultrafiltrated.





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### INTRODUCTION

Almost a third of the world's milk production is used in cheese making because cheese is a highly nutritious food and is an integral part of the daily consumed diet. Whereas, the production of white soft cheese in Egypt accounted for 75% of total cheese production in 2020 (European Union, 2019 and Farrag *et al.*, 2020). The number of research conducted on development of new types of soft cheese, which due to their technological and economical superiority in comparison to hard and pickled cheese has increased recently. In this regard, development of soft cheese is highly promising (Chechetkina *et al.*, 2016). Ultrafiltrated (UF) cheese is a very popular cheese group in the Mediterranean region. Low fat UF-cheese belong to dietetic products and represent a good base for creating products that may be classified as functional food (Miočinović *et al.*, 2011). During the past few decades over consumption of dietary fat is associated with various illnesses. This was the main reason to increase the demand for low-fat cheese.

Despite the variation of low-fat cheese in the market today, the successful manufacture of many full-flavored cheeses remains devious. However, the greater the reduction in fat, the greater is the challenges. The successful manufacture of low-fat cheese requires strict attention to many factors that impact flavor and body characteristics. In addition, the cheese maker has options of adding ingredients to produce cheese with desired firmness, mouthfeel, and flavor (**Johnson & Ibáñez, 2020**). Dairy processors have a great opportunity to solve the dilemma through the development of consumer acceptability to low fat cheese by supplementing low fat cheese with some additives with high nutritional and functional value, perhaps among of those additives are edible mushroom and bottle gourd seeds, as well as probiotic bacteria.

Mushrooms as a food supplements which are rich in very high quality protein, carbohydrates include fibers, minerals, vitamins, essential amino acids and unsaturated fatty acids. Moreover, several mushrooms have demonstrated efficient antibacterial activity as well as antifungal activity against resistant





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human pathogens (Khider *et al.*, 2017 and Shalaby *et al.*, 2018). The Oyster mushrooms (*Pleurotus ostreatus*) possess several medicinal properties including; anti-arthritic, antitumor, immune-modulatory, antioxidant, anticancer, anti-inflammatory, antigenotoxic, hypocholesterolaemic, anti-hyperglycaemic, anti-hypertensive, antiplatelet aggregating, antiviral and antimicrobial activities (Waktola & Temesgen, 2020).

On the other hand, bottle gourd (also known as Calabash and Molina with the scientific name *Lagenaria siceraria*) seeds proved to be one of the most important food supplements which contain a high percentage of oil (46.5 %) with high quality characteristics, oxidative stability and amounts of unsaturated fatty acids, also it is rich in omega 6 and 9. Also, it contains various phytochemical constituents, polyphenols and antioxidant activity. Moreover, bottle gourd seeds are rich in protein (10.75%), crude fibers (31.73%), carbohydrates (17.70%) and minerals. The seeds are edible and used in the fermented food products, fried cake, biscuits and pudding (Warra *et al.*, 2016; Atta *et al.*, 2020).

The past two decades have witnessed a global surge in the application of probiotics as functional ingredients in dairy industry in a number of dairy products including fermented milk (such as yogurt) and cheese. These probiotics are used as starter culture, where their presence imparts many functional characteristics to the product (for instance, improved aroma, taste, and textural characteristics) (Gao *et al.*, 2021). Consequently, the main objectives of the present work are to increase the vitality of low fat UF-soft cheese which supplemented with mushrooms or BGSP by adding probiotic bacteria.

# MATERIALS AND METHODS

#### 1. Materials

Skimmed ultra-filtrated buffalo's milk (retentate) was obtained from Dairy Processing Unit, belonging the Animal Production Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt. The ultrafiltration





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(UF) process was run using CARBO SEP UF unit (Type 2 S 151 Tubular, France) with Zirconium-Oxide membrane area of 6.8 m<sup>2</sup>, the inlet and outlet pressures were 5 and 3 bar, respectively. The concentration factor (C.F.) was 4 (w/w). The fresh Oyster mushroom (*Pleurotus ostreatus*) which used in the present study was obtained from Fungy Company for mushroom production, Giza, Egypt. Bottle gourd seeds were obtained from Cucurbits Research Department, Horticulture Research Institute, Dokki, Giza. *Bifidobacterium lactis* and *Lactobacillus acidophilus* were obtained from Dairy Microbiology Laboratory, National Research Center (NRC), Dokki, Giza, Egypt. Microbial rennet powder (CHY-MAX, 2280 IMCU/ml) was obtained from Chr. Hansen's Lab., Denmark. Food quality grade calcium chloride was obtained from El-Nasr Co., Cairo, Egypt. Dry fine edible grade sodium chloride (NaCl), produced by Egyptian salt and minerals company (EMISAL) was obtained from local market, Fayoum, Egypt. All chemicals that used in this study were analytical grade and purchased from El-Nasr, Sigma and Technogen Companies.

#### 2. Methods

#### 2.1. Preparation of Mushrooms

Fresh mushroom was cut and washed with hot, previously boiled water, then placed in an electric mixer for mashing and homogenization to form in a viscous suspension and then placed in the refrigerator until use.

#### 2.2. Preparation of bottle gourd seeds powder (BGSP)

Bottle gourd seeds were dehulled and milled to a fine powder with an electric grinder, then sifted, packed into polyethylene bags and stored in the refrigerator until use

#### 2.3. Activation of LAB strains

Both LAB strains (*Bif. lactis* and *Lb. acidophilus*) were activated first in MRS broth medium, then activated in 10 % (w/v) sterilized skim milk at 37°C for 24 hr. under microaerophilic conditions. Fresh activated cultures were used in this study.





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#### 2.4. Preparation of functional probiotic free fat UF- soft cheese

Two probiotic starters (*Lb. acidophilus* and *Bif. Lactis*) were used to make functional low fat UF- soft cheese according to **Maubois** *et al.* (1987). Each strain was used individually without additives or with adding fresh mushroom or BGSP as showed in **Fig.** (1). Samples of all resultant cheese treatments were analyzed for chemical composition when fresh and 28 days of storage at  $5\pm2^{\circ}$ C. While, microbiological and sensory attributes were determined when fresh, 7, 14, 21 and 28 d of storage at  $5\pm2^{\circ}$ C.



Fig. (1) : Schematic flow diagram of the basic steps involved in making functional probiotic free fat UFsoft cheese.

#### 2.5. Chemical analysis of resultant cheese and raw materials

The resultant cheese treatments, milk retentate, BGSP and mushroom samples were analyzed for their moisture (oven drying method), total nitrogen and





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water soluble nitrogen contents (macro Kjeldahal method), titratable acidity% (expressed as lactic acid), fat% (Gerber method), total dietary fibers and total ash contents (ashing at 550 °C) as described in A.O.A.C (2012). Salt (NaCl %) was determined by direct titration according to Bradley *et al.* (1992). The pH values were measured by using pH meter Thermo Scientific Orion Star (A 214).

#### 2.6. Microbiological examination

Enumerations of all microbial counts were done as described in **Oxoid** (2006). LAB including *Lb. acidophilus* and *Bif. lactis* in cheese samples were enumerated on MRS agar media and MRS agar supplemented with 0.5% L-cysteine hydrochloride, respectively. The total viable counts (TVCs) of cheese samples were determined using plate count agar medium. Fungi counts of cheese samples were determined on potato dextrose agar medium. Coliform bacterial counts of cheese samples determined on MacConkey agar medium.

#### 2.7. Organoleptic properties

The organoleptic properties of soft cheese samples were evaluated (at  $20 \pm 2^{\circ}$  C) during storage by 10 panelists of staff members of Dairy Science, Food Science & Technology Departments, Faculty of Agriculture, Fayoum University. Cheese samples were evaluated according to the score card sheet of **Hassan** *et al.* (1983), intervals storage period: fresh, 7, 14, 21 and 28 days. The total score (100 points) was divided into 50 points for flavor, 35 points for body & texture and 15 points for color and appearance.

#### 2.8. Statistical analysis

Data were statistically analyzed using General Linear Models procedure of Statistical Package for Social Sciences (**SPSS**, **2008**) Version 17.0.0 software. **Duncan's (1955)** multiple range tests were used to compare between the means.

#### **RESULTS AND DISCCUSION**

Cheese is a good dairy product for delivery of probiotic microorganisms into the human intestinal tract because of its specific chemical and physical





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characteristics compared to fermented milks (Karimi *et al.*, 2011). To be considered to offer probiotic health benefits, probiotics must remain viable in food products above  $10^6$  CFU/g until the time of consumption, without adversely altering sensory attributes (Pagano, 1998). Therefore, studying the influences of compositional and process factors (strains of probiotic bacteria, pH and titratable acidity, food additives, salt and storage period) affecting the viability of probiotics in this product as well as its sensory properties has been the subject of this study.

#### 1. Phesico-chemical analysis

#### 1.1. Chemical composition of raw materials

Chemical composition of fresh mushroom (FM), bottle gourd seeds powder (BGSP) and fresh free fat retentate) used for cheese manufacture are shown in **Table (1).** Analysis of retentate shows that moisture, fat, total protein and ash contents were 74, 0.60, 17.45 and 3.6%, respectively. Moreover, results showed that the BGSP contained 38% oil, 24.80% total protein, 3.24% ash, 4.00% fibers and 6.80% moisture. Close results about the chemical composition of BGS were reported by **Habibur Rahman (2003); Pallavi** *et al.* (2018) and Azab *et al.* (2021). This makes BGSP a good source of oil, protein and fibers with functional properties that are favorable for human consumption and industrial applications. It's clear from **Table (1)** that fresh mushroom (FM) contains high moisture content (86.00%) and low amount of fat, ash and fibers (0.22, 0.90 and 1.40%, respectively). Also, it contained 4.60% protein. These results are similar to which found by **Bach** *et al.* (2017) and **Galappaththi** *et al.* (2021).

Table (1	): Chemio	al compos	sition of	raw m	aterials
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Dow motorials					
Kaw materials	Moisture	Fat	protein	Fibers	Ash
Fresh free fat retentate	74.00	0.60	17.45	0.00	1.86
FM	86.00	0.22	4.60	0.90	1.40
BGSP	6.80	38.00	24.80	4.00	3.24





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#### 1.2. Gross chemical composition of probiotic free fat UF- soft cheese

The effects of adding different starter cultures (*Lb. acidophilus* or *Bif. lactis*) to free UF- soft cheese supplemented with FM or BGSP upon its chemical composition during the storage period are summarizes in **Table (2)**. The moisture results show that there were significant differences (P< 0.001) between treatments during storage at  $5\pm1$ °C for 28 days. Moisture content in all cheese treatments were slightly decreased during storage periods. This result was agreed with that obtained by **Degheidi** *et al.* (2009); Abd El-Salam (2015); Kebary *et al.* (2015) and Stankey *et al.* (2017). The results showed that fresh cheese treatments which contain fresh mushrooms with different starters (L<sub>2</sub> and B<sub>2</sub>) recorded higher moisture ratios (73.69 and 73.89%, respectively) than other treatments; this is due to the mushroom's high moisture content. Same results were found by Maray *et al.* (2017) and Mohammed *et al.* (2018). Whereas, the treatments containing BGSP had the lowest moisture percentages with different starters. Nearby results about the chemical composition of BGSP were reported by Habibur Rahman (2003), Pallavi *et al.* (2018) and Hassan *et al.* (2008).

According to the results obtained in the same Table, the variations in the fat content and fat/dry matter (F/DM) of cheese samples during storage were insignificant (P > 0.001), fat content in all cheese treatments slightly increased during storage because of moisture reduction. Also, it can be noticed that L<sub>3</sub> and B<sub>3</sub> (cheese treatments supplemented with BGSP) had higher fat content than control and cheese supplemented with mushrooms, it's because of the high fat content in BGSP. These results are in agreement with that stated by **Hassan** *et al* (**2008**). Bifido-cheese supplemented with 6% BGSP (B<sub>3</sub>) after 28 days contained the highest fat content (2.35%), while fresh probiotic control samples L<sub>1</sub> and B<sub>1</sub> recorded the lowest fat content of 0.90%. Results of F/DM had the same trend of fat content results. Fresh Bifido-cheese supplemented with 6% BGSP (B<sub>3</sub>) recorded the highest F/DM of 7.59%. The results indicate that moisture content and F/DM of all samples obtained Compatible with the CODEX legal standards





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for free fat soft cheeses **ES,2005**, where The proportion of F/DM should be is less than 10% and the moisture content does not exceed 75%.

It is evident from the obtained results that salt content (%) insignificantly increased during storage period in all cheese treatments, with an opposite trend of cheese moisture. The lowest percentage of salt was noticed in fresh Bifido-free fat cheese supplemented with 6% BGSP (B<sub>3</sub>) which contained 1.86% sodium chloride. While, the highest salt content was 2.22% for control cheese without any additives or starters at 28<sup>th</sup> d of storage.

Table (2): Chemical composition of functional free fat UF- soft cheese as affected by adding different starter cultures, BGSP, fresh Mushroom and storage periods at 5±1°C

Treatments		Storage		<b>(</b> 0)			
		Period (days)	Moisture	Fat	F/DM	Salt	Ash
С		Fresh	73.19 <sup>b</sup>	1.03	3.85	2.10	3.11 <sup>f</sup>
		28	69.66 <sup>de</sup>	1.16	3.84	2.22	3.37 <sup>d</sup>
	Τ1	Fresh	73.13 <sup>b</sup>	0.90	3.34	2.07	3.13 <sup>f</sup>
lus	LI	28	69.13 <sup>f</sup>	1.16	3.77	2.21	3.39 <sup>d</sup>
phi ese	тэ	Fresh	73.69 <sup>a</sup>	0.93	3.54	1.96	$3.50^{\circ}$
che		28	69.43 <sup>e</sup>	1.03	3.38	2.10	3.84 <sup>b</sup>
Aci	Т 2	Fresh	70.25 <sup>c</sup>	1.86	6.27	1.90	3.52 <sup>c</sup>
	LJ	28	66.74 <sup>h</sup>	1.96	5.91	1.98	3.94 <sup>a</sup>
e	D1	Fresh	73.24 <sup>b</sup>	0.90	3.36	2.03	3.23 <sup>e</sup>
ees	DI	28	69.51 <sup>e</sup>	1.16	3.82	2.18	3.53°
ch	DJ	Fresh	73.89 <sup>a</sup>	0.93	3.57	1.90	3.41 <sup>d</sup>
dic	j B2	28	69.86 <sup>d</sup>	1.08	3.59	2.08	3.85 <sup>b</sup>
3ifi	iji R2	Fresh	70.16 <sup>c</sup>	2.26	7.59	1.86	3.52 <sup>c</sup>
<b>H</b> D3	28	67.21 <sup>g</sup>	2.35	7.16	1.93	3.97 <sup>a</sup>	
	SE±		0.06	0.04	0.17	0.03	0.02
Sig.			***	NS	NS	NS	***

a, b,.... and j: Means having different superscripts within each column are significantly different (p < 0.001).

C: control, free-fat UF- soft cheese without BGSP or mushroom

 $L_1$ : free-fat UF- soft cheese inoculated with 1% Lb. acidophilus.

L<sub>2</sub>: free-fat UF- soft cheese supplemented with 5 % fresh mushroom and 1% Lb. acidophilus.

L<sub>3</sub>: free-fat UF- soft cheese supplemented with 6 % BGSP and 1% *Lb. acidophilus.* 

**B**<sub>1</sub>: free-fat UF- soft cheese inoculated with 1% *Bif. Lactis.* 

B2: free-fat UF- soft cheese supplemented with 5 % fresh mushroom and 1% Bif. Lactis.

B<sub>3</sub>: free-fat UF- soft cheese supplemented with 6 % BGSP and 1% Bif. Lactis.

SE: Standard error Sig.: Significance NS: Not significant





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Results in **Table (2)** showed the ash content of UF-white soft cheese treatments during storage. As clear from results, there was a significant difference ( $P \le 0.001$ ) between UF-white soft cheese treatments and during storage period. The ash values increased during storage period in all experimental cheese, with an opposite trend to the cheese moisture. These results were in harmony with those of **Metry** *et al.* (2017). The highest percentages of ash were in treatments supplemented with BGSP ( $L_3$  and  $B_3$ ) at 28<sup>th</sup> d where; it was 3.94 and 3.97%, respectively. It is due to the high ash content of BGSP. Results are in agreement with that stated by **Hassan** *et al.* (2008). Fresh control treatment contained the lowest percentage of ash; being 3.11%. While, the lowest ash content was 3.11% for fresh control cheese (C).

# 1.3. Total nitrogen, water soluble nitrogen and water soluble nitrogen/total nitrogen

Generally, there was a slightly significant (P<0.001) increase in total nitrogen (TN%) during storage period in all treatments. The highest percentage of TN (2.84%) was for sample (L<sub>3</sub>) which contains 6% BGSP and starter *Lb. acidophilus*; this could be attributed to the fact that BGSP contain a high percentage of protein. These results were in line with those reported by **Ibeabuchi, J. C. (2014).** Similar trends were obtained by **Mehaia (2002); Elewa** *et al.* (2009); Sudhir *et al.*, 2010; Kebary *et al.* (2015) and Abd El-Salam (2015).

In cheese, water soluble nitrogen (WSN %) is regarded as a measure of proteolysis during storage period. Results **Fig. (2)** showed that acidophilus UF-soft cheese without additives (L<sub>1</sub>) and which supplemented with fresh mushroom (L<sub>2</sub>) at the end of storage period were recorded the significantly (P<0.001) highest WSN% of 0.96 and 0.97%, respectively. While, the lowest percentage was 0.59% for fresh control cheese. Moreover, WSN% is commonly reported as percentage of the TN %. The results obtained in **Fig. (2)** explained WSN/ TN (%) of UF-white soft cheese samples produced from different treatments during storage period. The highest value of WSN/TN % was 36.02% for L<sub>1</sub> which didn't





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contained any additives but a starter culture of *Lb. acidophilus*, it may due to the count of mesophilic bacteria and psychrotrophic bacteria is an indicators of microbial proteolysis. Similar results were mentioned by **Chramostová** *et al.* (2016).While, fresh treatments contained BGSP with both probiotic starters ( $L_3$  and  $B_3$ ) recorded significantly lowest proteolysis indicated by WSN/TN % (22.65 and 22.67, respectively). This result may due to the antibacterial activity of bottle gourd seeds. it's in agreement with **Nagaraja** *et al.* (2011) who reported that bottle gourd seeds have a moderate to potent antimicrobial activity against the bacterial strains: *Escherichia coli, Enterococcus faecalis, Klebsiella pneumonia, Salmonella typhi, Staphylococcus aureus* and antifungal strains: such as *Aspergillus flavus, Aspergillus oryzae* and *Trichoderma harzianum*.



Fig. (2): Changes in TN%, WSN% and WSN/TN% content of free fat UF- soft cheese during storage periods at  $5\pm1^\circ C$ 





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#### **1.4.** The pH values and titratable acidity (TA%)

The pH values and titratable acidity of functional low fat UF- soft cheese were affected significantly ( $P \le 0.001$ ) by adding different probiotic starters with fresh mushrooms or BGSP and storage periods at  $5\pm1^{\circ}$ C (Fig. 3). The noncultured control cheese (C) recorded the highest pH values when fresh (6.76), at 7 d (6.72) and till 14 d of storage (6.64) without significant differences in between. Whereas, the lowest pH value was for L<sub>2</sub> (Acidophilus UF- free fat soft cheese supplemented with fresh mushrooms|) after 28 d (5.72). It can be noticed from results that pH values of treatments which inoculated with *Lb. acidophilus* (L<sub>1</sub>, L<sub>2</sub> and L<sub>3</sub>) were higher than its equivalents which cultured with *Bif. lactis* (B<sub>1</sub>, B<sub>2</sub> and B<sub>3</sub>), the first starter produce an amount of lactic acid more than the later as a result of fermenting lactose during metabolism process. Results are in harmony with that stated by **Moayednia & Mazaheri (2013).** 



Fig. (3): Changes in pH values and titratable acidity of functional free fat UF- soft cheese





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On the other hand, significant ( $P \le 0.001$ ) increases of acidity occurred along with the significant decreases of the pH values during the storage period in all assessments. The highest TA% was 0.95% for Acidophilus UF- free fat soft cheese supplemented with fresh mushrooms (L<sub>2</sub>) after 28 d of storage. While, the fresh control cheese had the significantly lowest TA of 0.42%. The increase in TA% and the decrease in pH values during storage may due to the viability of probiotic starters at early storage period. Moreover, these changes in TA% and pH associated with high numbers of total viable counts at the end of storage. These results were in agreement with **Nasr et al.** (2018).

#### 2. Microbiological examination of free UF- soft cheese.

#### 2.1. Viability of probiotic starters

Probiotics are defined as "live microorganisms which when administered in adequate numbers confer a health benefit on the host" (FAO/WHO, 2001). Probiotic bacteria, specifically bifidobacteria and lactobacilli, are the normal inhabitants of the human colon. To provide health benefits related to probiotic organisms, the minimum viable counts of each probiotic strain in gram or milliliter of probiotic products should be more than  $10^6$  CFU/g at the time of consumption for probiotic bacteria as reviewed by Karimi *et al.* (2011). It's clear from Fig. (4) that all cheese treatments except control (C, UF-soft cheese without probiotic starter or additives) contained more than  $1 \times 10^6$  CFU/g when fresh and till 21 d of cold storage. Only probiotic cheese treatments supplemented with BGSP with *Lb. acidophilus* or *Bif. lactis* (L<sub>3</sub> and B<sub>3</sub>, respectively) can be considered probiotic products at 28 d, it may be due to prebiotic activity as gourd seeds are known to present high dietary fiber contents with prebiotic activity (Sreenivas & Lele, 2013; Pallavi *et al.*, 2020 and Atta *et al.*, 2020).

Moreover, Results showed that the probiotics' viability was significantly ( $P \le 0.001$ ) affected by different treatments and storage periods. The highest probiotic count was  $59.93 \times 10^6$  CFU/g which recorded by bifido-cheese supplemented with BGSP (B<sub>3</sub>) at 14 d of storage. While, the significantly lowest numbers were for non-inoculated control cheese (C) and fresh probiotic cheese





treatments without additives ( $L_1$  and  $B_1$ ) when fresh and at the end of storage period. It's clear form **Fig. (4)** that the best time to consume all probiotic cheese treatments is till 14 d of cold storage, where the probiotic's viability is at the max.



Fig. (4): Changes in LAB content of free fat UF-white soft cheese during storage at 5±1°C

#### 2.2. Total viable counts (TVCs), Yeast & mold and coliform bacterial count

The total viable count (TVC) of UF-white soft cheese treatments was significantly ( $P \le 0.001$ ) affected by different treatment and storage periods (**Fig. 5**). In all cheese treatments, the TVC gradually increased during storage period. The lowest numbers of TVC were for fresh UF-soft cheese without any additives such as C, L<sub>1</sub> and B<sub>1</sub> (0.30, 0.63 and  $0.33 \times 10^4$  CFU/g, respectively). While the highest number observed in control cheese at the end of the storage period with TVC of  $109.53 \times 10^4$  CFU/g. these results are in harmony with pH and TA% results, the pH values decreased as long as TVC increased in cheese samples. These results were similar to obtained by **Metry (2010)** and **Nasr et al. (2018).** 

Yeasts and molds were not detected in all treatments and control at fresh samples, but it were detected at 28 days of storage with numbers of 2.3, 2.1, 3.2, and 2, 2.7 CFU/g for C,  $L_1$ ,  $L_3$ ,  $B_1$  and  $B_3$  treatments, respectively. Also, coliform group was not detected in all UF-soft cheese samples either when fresh or during cold storage period (28 d). This might be due to the efficient heat treatment of milk which inhibits the vegetative cells, also the sanitation and hygienic conditions during the manufacture process of UF-white soft cheese and cold





storage. These results were in agreement with Metry (2010); Abd El-Salam *et al.* (2015); Awad *et al.* (2015) and Azab *et al.* (2021).



Fig. (5): Changes in TVC of free fat UF-white soft cheese during storage at 5±1°C

#### 3. Organoleptic properties

The organoleptic properties of UF- white soft cheese as affected (main effects) by various additives (fresh mushrooms and BGSP), different starter cultures and cold storage period are shown in **Table (3)**. From these results, it is easy to observe that acidophilus and bifido UF-soft cheese supplemented with fresh mushrooms (L<sub>2</sub> and B<sub>2</sub>, respectively) had significantly higher scores of flavor, body & texture and total score than all other treatments. While, control cheese (C) recorded the highest score for color & appearance of 15 points. It means that fresh mushroom was more organoleptically acceptable by the panelists, regardless different storage periods. The significantly (P  $\leq$  0.001) lowest total scores were 92.66 and 91.33 points for acidophilus and bifido UF-soft cheese without any additives (L<sub>1</sub> and B<sub>1</sub>, respectively). On the other hand, results of storage period main effect illustrated that body & texture and color & appearance were insignificantly (P  $\geq$  0.001) affected. Whereas, storage periods had a significant (P  $\leq$  0.001) effect on flavor and total scores. It can be concluded from results that it's better to consume probiotic cheese when fresh and till 14 d of storage.

The effect of interaction between different treatments and storage periods on the organoleptic properties of UF-soft cheese was displayed in **Fig. (6).** Results





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showed that all organoleptic properties and total score were significantly (P > 0.001) affected while; the interaction effect on cheese flavor was insignificant. It can be noticed that scores of all properties in all treatments decreased after 14 days of storage reaching the lowest scores at 28 days. The highest total score was 99.00 points, recorded by acidophilus UF-soft cheese supplemented with fresh mushroom (L2) at 14th day of storage while, the lowest total score (87.33 points) was for treatment B1 (Bifido UF-soft cheese without additives) at the end of storage period. These results may be attributed to the analytic activity of different probiotic bacteria which can improve flavor and texture by increasing proteolysis and the presence of mushroom and BGSP used in the manufacture of UF white soft cheese because they contain many ingredients that improve the flavor and texture of the cheese.

Effect	Flavor (50)	Body and texture	Color & Appearance (15)	Total score
	(50)		Appearance (15)	(100)
С	$46.33^{d}$	32.53 <sup>b</sup>	$15.00^{a}$	93.86 <sup>c</sup>
L <sub>1</sub>	47.13 <sup>cd</sup>	32.00 <sup>b</sup>	13.53 <sup>cd</sup>	92.66 <sup>d</sup>
$L_2$	49.33 <sup>a</sup>	$34.20^{a}$	$14.40^{b}$	97.93 <sup>a</sup>
$\tilde{L_3}$	$47.80^{\circ}$	34.06 <sup>a</sup>	13.80 <sup>c</sup>	95.66 <sup>b</sup>
$\mathbf{B}_{1}$	46.06 <sup>e</sup>	32.13 <sup>b</sup>	13.13 <sup>d</sup>	91.33 <sup>d</sup>
$\mathbf{B}_2$	48.93 <sup>ab</sup>	33.86 <sup>a</sup>	$14.40^{b}$	$97.20^{a}$
$\mathbf{B}_{3}$	48.06 <sup>bc</sup>	33.93 <sup>a</sup>	13.60 <sup>cd</sup>	$95.60^{b}$
<b>SE</b> ±	0.35	0.25	0.19	0.52
Sig.	***	***	***	***
		Storage period (d	lays)	
Fresh	48.52a	33.57	13.81	$95.90^{a}$
7	$48.28^{ab}$	33.47	13.90	95.66 <sup>a</sup>
14	47.57 <sup>bc</sup>	33.28	13.85	94.71 <sup>ab</sup>
21	47.04 <sup>c</sup>	32.76	14.38	94.19 <sup>b</sup>
28	46.90 <sup>c</sup>	33.14	13.95	$94.00^{b}$
SE±	0.29	0.21	0.16	0.43
Sig.	***	NS	NS	**

 Table (3): Scores of sensory evaluation for functional free fat UF-soft cheese as affected by adding different ratios of LAB, BGSP, mushroom and storage periods (main effects).

a, b and c: Means having different superscripts within each column are significantly different (p <0.001).

\* See Table (2), SE: standard error.





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Fig (6): Changes in (a) flavor, (b) body and texture, (c) color and (d) total score of low UF-white soft cheese during storage at 5±1°C





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# CONCLUSION

It can be concluded from results of this study that adding probiotic bacteria (*Lb. acidophilus* or *Bif. lactis*) to functional UF- soft cheese supplemented with FM or BGSP make that cheese a suitable carrier for the probiotics into the human intestine as well, to improve its vitality and quality. Bifido-cheese supplemented with BGSP ( $B_3$ ) at 14 days of storage had the highest probiotic viability where, both Acidophilus and Bifido-free fat soft cheese supplemented with 5% FM had the best sensory properties. Therefore, it is recommended to produce Acidophilus and Bifido free fat UF-soft cheese supplemented with 5% FM or 6% BGSP as a new vital product with healthy properties.

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