



American Journal of  
**Food Technology**

ISSN 1557-4571



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## Research Article

# Tallaga Cheese as a New Functional Dairy Product

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### Abstract

**Background and Objective:** Probiotic functional dairy products are known of their positive impact on overall health. Also, mushroom has a healthy magical effect on human health. So, a new style Tallaga cheese which prepared by using some strains of probiotic bacteria and different ratios of mushroom was manufactured. Screening of some strains of probiotic bacteria for their antibacterial activity was applied. **Methodology:** Fresh buffalo milk samples were standardized to 4% fat, pasteurized, cooled to 37°C, inoculated with starter culture as well as probiotic bacteria *Lactobacillus reuteri* B-14171 at the same ratio (1%), salted and fortified with different ratios of mushroom to achieve four treatments. The first treatment was prepared without mushroom (control), while the other three treatments fortified with 3, 5 and 10% (w/v) mushroom to present T1, T2 and T3, respectively. **Results:** All milk cheese were turned to Tallaga cheese and stored at 7±2°C for 30 days. The fresh samples were analyzed for their gross composition; all stored samples were evaluated for their organoleptic properties, microbiology quality and some chemical parameters when fresh and after 7, 15 and 30 days at 7±2°C. **Conclusion:** Data revealed that adding mushroom up to 3% increased Total Bacterial Count (TBC), probiotic and lactic acid bacteria, while coliform group and mould and yeast were decreased. Mushroom had been pronoucnly improved the sensory properties of resultant cheese especially in treatment (T1). It could be concluded that using of 3% mushroom and 1% *L. reuteri* B-14171 succeeded in prepared functional Tallaga cheese.

**Key words:** Probiotic bacteria, mushroom, functional Tallaga cheese, antimicrobial activity

**Received:** April 06, 2016

**Accepted:** June 15, 2016

**Published:** August 15, 2016

**Citation:** W. El-Kholy, Azzat B. Abd El-Khalek, Sahar H.S. Mohamed, Mohamed T. Fouad and Jihan M. Kassem, 2016. Tallaga cheese as a new functional dairy product. Am. J. Food Technol., 11: 182-192.

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The importance of food as a reason for improving health has suggested a new division of food, namely functional food<sup>1</sup>. The demand for functional food is growing rapidly all over the world due to increase awareness of the consumers on the impact of food on health<sup>2</sup>. Functional food means food possessing important levels of biologically active components which give certain health benefits, more than the traditional nutrients that they have<sup>3</sup>.

Probiotic products are actually significant functional food, because they represent about 65% of the world functional food market<sup>4</sup>. Probiotic bacteria have been including into a wide variety of foods, like dairy products (such as yogurt, cheese) and also in non-dairy products such as juices and cereals<sup>5</sup>. Some health benefits for product that contain live probiotic bacteria were claimed, including reducing of symptoms of lactose intolerance, treatment of diarrhea, anti-carcinogenic properties, reduction in blood cholesterol, improvement in immunity, food allergies, intestinal infection, constipation gastroenteritis, hepatic, flatulence, colitis, gastric acidity and osteoporosis<sup>6-9</sup>.

Nowadays probiotic bacteria used in commercial products are members of the genera *Lactobacillus* (such as *L. acidophilus*, *L. reuteria* and *L. rhamnosus*) and *Bifidobacterium*<sup>10</sup>. Dairy products which contain probiotic bacteria were on the market all over the world such as Sweden, Finland, Japan and Switzerland<sup>11</sup>. However, probiotic bacteria can be added with starter cultures during manufacturing of dairy products<sup>12,13</sup>.

On the other hand, mushrooms have been used for thousand years as food and for medicinal purposes. It is clear that the increased cultivation of mushrooms can support in resolving many problems of global importance such as protein insufficiency as well as amelioration the health. Mushrooms are valuable health foods which are low in calories and supply with essential minerals<sup>14</sup>. Mushrooms are an excellent source of B vitamins, including riboflavin and pantothenic acid that provide energy by breaking down proteins, fats and carbohydrates. The B vitamins play a significant role in the nervous system, maintain healthy red blood cell and keeps health skin and make sure the digestive and nervous systems function properly. Mushrooms are also considered a source of important minerals like selenium, which works as an antioxidant that protect body cells from damage that can lead to heart disease, some cancers (such as breast and prostate cancer) and other

diseases of aging, ergothioneine copper, potassium and  $\beta$ -glucans. Copper plays a role in making red blood cells that carry oxygen through the body. It also maintains bones and nerves healthy. Potassium is a significant mineral that keeps normal fluid and mineral balance that controls blood pressure<sup>15</sup>.

Cheese is the greatest part of the dairy market in Egypt; its percentage is approximately 40% of the total market value. Soft cheese grew quickly in 2012, at a rate of 26% in terms of current value and 15% in terms of volume. In Egypt, traditional dinner usually contains a piece of white cheese and bread<sup>16</sup>.

Tallaga cheese is the most popular local type of package or unpackaged soft cheeses by all socioeconomic classes in Egypt due to its nutritional value, convenience and good taste, clean pleasant creamy low salty taste with a spreadable mellow soft body. It is a product closely related to Domiati cheese and mainly ready for consumption within one month of storage at refrigerator temperature<sup>17-19</sup>.

Fresh soft cheese seems to be a perfect means for carrying probiotic bacteria and mushroom, to act as a new functional dairy product, as it contains high moisture and relatively low salt contents, stored at 4°C. Therefore, the major target of this research is applying some strains of probiotic bacteria with different ratios of mushroom for preparation of new style functional Tallaga cheese and investigated the chemical, microbiological and organoleptic properties of the resultant cheese.

## MATERIALS AND METHODS

### Materials

**Milk:** Fresh raw buffalo's milk was standardized to 4% fat. It was bought from faculty of Agriculture, Cairo University, Egypt.

**Mushroom:** Fresh mushroom was purchased from great super markets in Cairo, Egypt.

**Rennet:** Hannilas rennet powder was bought from Chr-Hansen's Lab, Denmark (CHY-Max powder extra).

**Salt:** A commercial fine grade salt was bought from El-Nasr saline's company, Egypt.

**Calcium chloride:** It was purchased from Sigma Company, USA.

**Starter cultures and probiotic bacteria:**

- *Lactococcus lactis* subsp., *cremors* and *Lactococcus lactis* subsp., *lactis* were obtained from Dairy Microbiology Laboratory, National Research Centre
- *Lactobacillus plantarum* Dsaz 0174 was obtained from Cairo Microbiology Resources Center (Cairo-Mircen), Faculty of Agriculture Ain Shams University
- *Lactobacillus acidophilus* N4495 was obtained from Chr. Hansen Laboratores, Copenhagen, Denmark
- *Lactobacillus rhamnosus* Tistr 541 was brought from Thailand institute of Scientific and Technological Research, Bangkok, Thailand
- *Lactobacillus reuteri* B-14171 and *Lactobacillus gasserii* (subgroup B<sub>1</sub> of the *acidophilus* group) strain B-14168 were provide by Northern Regional Research Laboratory illions USA (NRRL)
- Starter cultures and probiotic bacteria strains were activated in 11% sterilized retrieved skim milk and incubated at 30 or 37°C, respectively for 24 h

**Pathogenic bacteria strains (indicators):** *Escherichia coli* 0157: H7 ATCC 6933, *Bacillus cereus* ATCC 33018, *Staphylococcus aureus* ATCC 20231, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027 and *Listeria monocytogenes* V7 as reference strains were obtained from the stock cultures of the Agricultural Research Centre in Giza. All strains were routinely maintained by sub-culturing once a week in tryptone soya broth/agar and stored at 4°C until use.

**Methods**

**Preparation of mushrooms:** Fresh mushrooms were thoroughly washed with run water then cut into small pieces

by sharp knife and boiled in hot water at 100°C for 5 min, cold and stored in refrigerator at 7±2°C until use.

**Tallaga cheese manufacture:** Tallaga cheese making was carried out according to Abdel-Salam<sup>1</sup> and modified with addition of different amounts of mushroom (Fig. 1). Fresh buffalo’s milk was heated at 72°C for 15 sec, cheese milk was immediately cooled to 37°C. Starter cultures and probiotic strain were added at level of 1%. Calcium chloride, sodium chloride and rennet were added at the ratios of 0.02, 3 and 0.05% (w/v), respectively. The milk was divided into 4 equal portions: (1) Starter cultures (*Lactococcus lactis* and *Lactococcus cremoris*) and probiotic (*Lactobacillus reuterii*) (served as a control), (2) Starter cultures+probiotic+3% mushroom (T<sub>1</sub>), (3) Starter cultures+probiotic+5% mushroom (T<sub>2</sub>) and (4) Starter cultures+probiotic+10% mushroom (T<sub>3</sub>). All cheese milk was kept to coagulate at room temperature.

The resultant cheese was traditionally completed, the curd was whey out and the cheese were packed in plastic containers and stored in refrigerator at 7±2°C. Trials were analyzed when fresh and after 7, 15 and 30 days.

**Detection of antimicrobial activity of probiotic bacteria**

**used:** Probiotic bacteria strains cultivated in MRS broth (37°C for 18 h). The cell-free supernatant fluids were obtained by centrifuging MRS broth cultures at 4000 rpm for 15 min at 4°C. Supernatant liquids were sterilized using filter membranes 0.22 µm Millex-GV (Millipore) and pH adjusted to 6 with 2N NaOH. The activity of resulting supernatant were tested against pathogenic bacteria (indicator strains) using agar well diffusion method as described by Lyon *et al.*<sup>20</sup>. The indicator strains were freshly diluted in brain heart infusion broth and 100 µL of each were plated onto nutrient agar plates. Plates were air dried for 15 min and the wells (5 mm

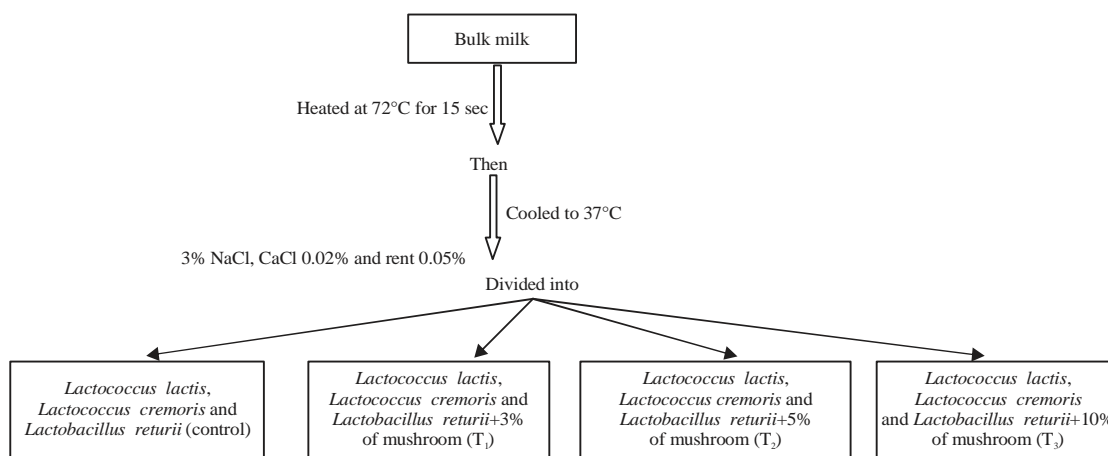


Fig. 1: Tallaga cheese making was carried out according to Abdel-Salam<sup>1</sup> and modified with addition of mushroom

diameter) were done and filled with 30 µL of cell-free supernatant fluids samples, then incubation was done at 37°C for 24 h, the inhibition zones diameter around the wells caused by the diffusing metabolites (antimicrobial substance) were measured in millimeters. The control inoculated plates were prepared as the same described but with MRS broth without antimicrobial substance samples. All assays were done in duplicate and the results presented were the mean of duplicate trails.

### Analytical procedure

**Microbiological analysis:** Cheese samples (25 g) each was homogenized for 1 min with 225 mL of sterile solution (2% w/v) of sodium citrate.

Methods of microbiological analysis were followed according to APHA<sup>21</sup> for:

- Total bacteria count on plate count agar (oxiod)
- Total coliform bacterial on violet red bile agar (oxiod)
- Mould and yeast on acidified potato dextrose agar (oxiod)
- Lactic acid bacteria (starter cultures) (*Lactococcus* strains) were counted on M17 agar (oxiod) after aerobic incubation at 30°C for 48 h
- Probiotic strains (*Lactobacillus reuteri*) were enumerated on MRS-arabinose agar

The MRS basal medium was prepared without dextrose and 10 mL of membrane filtered sterile solution of 10% L-arabinose was added to 20 mL of basal medium (1% final concentration) just before pouring the agar medium. Plates were an aerobically incubation at 37°C for 48 h<sup>22</sup>.

**Chemical analysis:** Fresh samples of Tallaga cheese were chemically analyzed for their Total Solids (TS), fat (%), crud fiber and Total Protein (TP) contents according to AOAC<sup>23</sup>. Stored samples were periodically analyzed when 0, 7, 15 and 30 days for pH value using digital pH meter (HANNA, Instrument, Portugal) with glass electrode.

At the same time, total ash of fresh samples of Tallaga cheese was also determined according to Raghuramulu *et al.*<sup>24</sup>.

The content of the available carbohydrate was determined by Raghuramulu *et al.*<sup>24</sup> for fresh Tallaga cheese samples.

**Acetaldehyde content:** The acetaldehyde content was estimated for stored Tallaga cheese samples as described by Lee and Jago<sup>25</sup> using the conway microdiffusion semi-carbozide methods.

**Diacyetyl content:** The previous procedure for determination of the acetaldehyde content was used also to determine diacyetyl content with the exception of measuring the absorption at 270 nm. The same spectrophotometer was used as described by Lee and Jago<sup>25</sup>.

**Organoleptic properties:** Organoleptic properties of cheese samples were evaluated according to the suggesting of Keating and White<sup>26</sup> when 0, 7, 15 and 30 days of cold storage by 10 expert panelists of members of Dairy Department, National Research Center. Panelists evaluated cheese for appearance (10 points), body and texture (40 points) and flavor (50 points).

**Statistical analysis:** Statistical analysis was achieved using Statistical Package for Social Studies Software SPSS<sup>27</sup>. Significance of difference of various groups was determined by the LSD (least significant difference) test ( $p \leq 0.05$ ). Complete randomized design was used to estimate chemical, microbiological and sensory characteristics of Tallaga cheese.

## RESULTS AND DISCUSSION

**Antibacterial activity of probiotic bacteria:** Results recorded in Table 1 revealed that all probiotic bacteria examined have variable antibacterial activity against all of the tested pathogenic bacteria. *Lactobacillus reuteri* was the most effective probiotic culture among all probiotic strains tested against pathogenic bacteria. From the Table 1, *Lactobacillus reuteri* showed strongly inhibitory activity towards *B. cereus*, *E. coli* O157:H7, *Staphylococcus aureus* and *P. aeruginosa* as the most sensitive indicators with the diameter zones of 30, 23, 22 and 20 mm, respectively. While, *L. monocytogenes* and *S. typhimurium* were less sensitive with the diameter zone 17 and 15 mm, respectively. They followed by *Lactobacillus rhamnosus*, these results were in agreement with Lewus *et al.*<sup>28</sup> and Alak *et al.*<sup>29</sup>

Table 1: Antibacterial activity of probiotic cultures against pathogenic and spoilage bacteria

Pathogenic bacteria	Probiotic bacteria				
	1	2	3	4	5
	<b>Diameter of inhibition zone (mm)</b>				
<i>Salmonella typhimurium</i>	10	15	11	13	9
<i>E. coli</i> O157:H7	17	23	15	18	10
<i>Pseudomonas aeruginosa</i>	10	20	13	17	11
<i>Listeria monocytogenes</i>	15	17	11	15	13
<i>Bacillus cereus</i>	13	30	18	10	10
<i>Staphylococcus aureus</i>	10	22	12	13	12

1: *L. plantarum*, 2: *L. reuteri*, 3: *L. acidophilus*, 4: *L. rhamnosus* and 5: *L. gasseri*

who observed that *L. reuteri* inhibited the growth of other bacteria by the production of inhibitory substance (reuterin), a bacteriocin which has antimicrobial activity against pathogenic bacteria such as *Escherichia* species, also the obtained results in this study had been supported by the finding published by Jacobsen *et al.*<sup>30</sup> who reported that the antagonistic activity is possibly due to the antibacterial substance produced by probiotic strains.

Several *Lactobacillus* strains have been found to produce various types of antibiotic, *L. acidophilus* produces lactacin, while *L. plantraum* produces plantaricin<sup>31,32</sup>. *Lactobacillus* also produces hydrogen peroxide and organic acid such as lactic and acetic acids, which inhibit growth of many pathogenic gram negative organisms<sup>33</sup>. A similar effect was also reported by El-Ziney and Debevere<sup>34</sup> and El-Ziney *et al.*<sup>35</sup>. Olasupo *et al.*<sup>36</sup> assessed bacteriocin producing *Lactobacillus* strains from fermented food is active against enterotoxigenic *E. coli*, *S. aureus*, *S. typhimurum* and *Pseudomonas* sp. Dabiza *et al.*<sup>37</sup> isolated 200 strains of lactic acid bacteria from dairy product samples. They found a high proportion of these strains lactobacilli exhibited broad spectrum of antagonistic against spoilage and pathogenic bacteria, they added that the cell free supernatant of *L. rhamnosus* showed the highest effect among lactobacilli against *E. coli* 0157:H7, *S. aureus*, *B. cereus* and *Aeromonas hydrophila*. These observations were in the line with those of Vescovo *et al.*<sup>38</sup> and El-Shafei *et al.*<sup>39</sup>. Therefore, *Lactobacillus reuteri* was selected for Tallaga cheese making.

**Microbiological analysis:** Microbial analysis (total viable bacterial counts, coliform group, mould and yeast, probiotic bacteria and starter culture) of Tallaga cheese presented in Fig. 2-6, respectively. Data showed that no significant

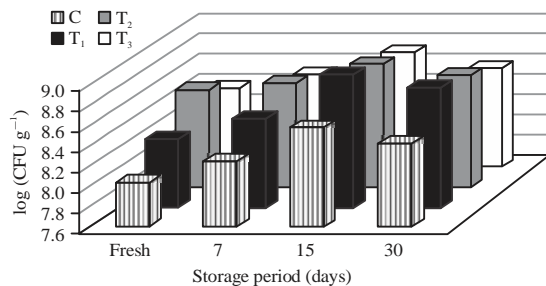


Fig. 2: Total bacterial counts (CFU g<sup>-1</sup>) in Tallaga cheese fortified with 3, 5 and 10% of mushroom during cold storage period for 30 days, C: Control, T<sub>1</sub>: 3% mushroom, T<sub>2</sub>: 5% mushroom and T<sub>3</sub>: 10% mushroom

difference between control and cheese treatments in the total viable bacterial counts (p>0.05). An insignificant increase was observed by adding the mushroom to the cheese if compared with control. However, the counts gradually increased till the first 15 days of cold storage period, then slightly decreased at the end of storage time Fig. 2. The result of Tallaga cheese made with 3 and 5% mushroom had slight increase in total

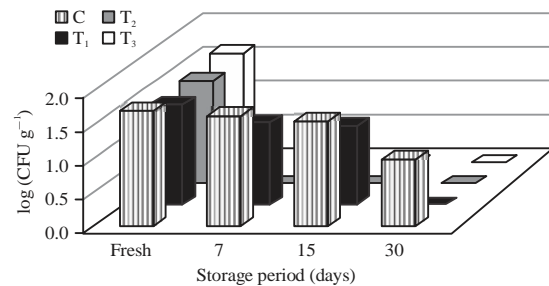


Fig. 3: Coliform bacteria counts CFU g<sup>-1</sup> in Tallaga cheese fortified with 3, 5 and 10% of mushroom during cold storage period for 30 days, C: Control, T<sub>1</sub>: 3% mushroom, T<sub>2</sub>: 5% mushroom and T<sub>3</sub>: 10% mushroom

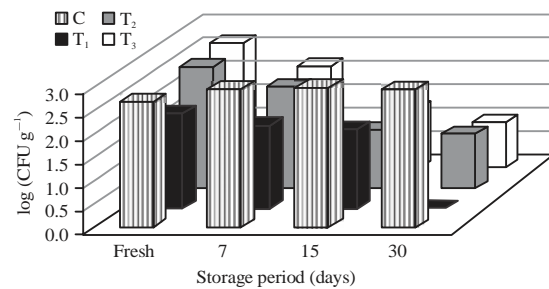


Fig. 4: Mould and yeast counts log CFU g<sup>-1</sup> in Tallaga cheese fortified with 3, 5 and 10% of mushroom during cold storage period for 30 days, C: Control, T<sub>1</sub>: 3% mushroom, T<sub>2</sub>: 5% mushroom and T<sub>3</sub>: 10% mushroom

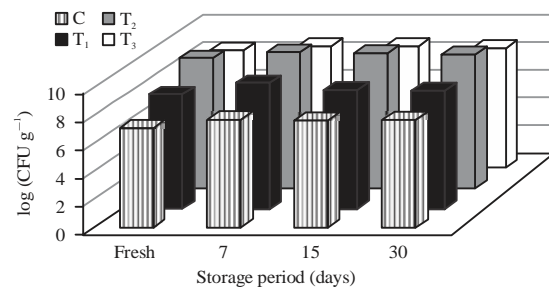


Fig. 5: *Lactobacillus reuteri* counts CFU g<sup>-1</sup> in Tallaga cheese fortified with 3, 5 and 10% of mushroom during cold storage period for 30 days, C: Control, T<sub>1</sub>: 3% mushroom, T<sub>2</sub>: 5% mushroom and T<sub>3</sub>: 10% mushroom

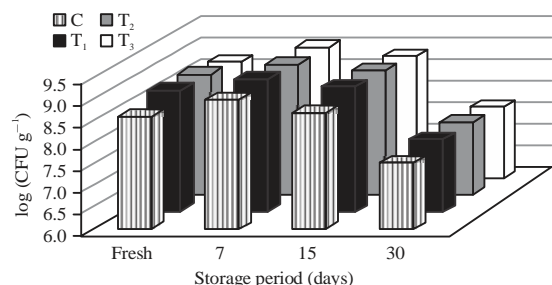


Fig. 6: Lactic acid bacteria counts log CFU g<sup>-1</sup> in Tallaga cheese fortified with 3, 5 and 10% of mushroom during cold storage period for 30 days, C: Control, T<sub>1</sub>: 3% mushroom, T<sub>2</sub>: 5% mushroom and T<sub>3</sub>: 10% mushroom

viable bacterial counts (log<sub>10</sub> CFU 8.79 and 8.72, respectively) if compared with control (log<sub>10</sub> CFU 8.43). The results coincided with those stated by other investigators<sup>22,40</sup>. The out results were in agreement with Abou-Zeid<sup>41</sup> who found that the total bacterial count in domiati cheese made with parsley/rocket was higher than in control cheese. The same results were in agreement with El-Kholy<sup>42</sup> and Abdeen<sup>43</sup>, they reported that during the cold storage of cheese, the total bacterial counts slightly increased during the first period of storage and then gradually decrease till the end of cold storage period at 5 °C. That decrease would be evidentially referred to the increase in titratable acidity which control rate of bacterial growth<sup>40</sup>. This result indicated that adding mushroom in cheese milk had stimulatory effect on microbial flora in functional Tallaga cheese and the stimulation was apparent in fresh cheese until reached to a maximum rate at 15 days of cold storage period.

Regarding the coliform bacterial group, data in Fig. 3 showed clearly that coliform was presented in both control cheese and functional Tallaga cheese made with adding mushroom at 3, 5 and 10% concentration. Control cheese had significantly ( $p < 0.05$ ) higher number of coliform count during storage period if compared with cheese treatments, this is obviously due to recontamination and suppressive effect of added starter cultures and probiotic *L. reuteri* on coliform bacterial. During cold storage period, coliform counts decreased rapidly in all samples to undetected after 7 days for treatments T<sub>2</sub> and T<sub>3</sub> (5 and 10% mushroom respectively) and after 15 days for treatment T<sub>1</sub> (3% mushroom), while coliform group was presented in control cheese until the end of cold storage period Fig. 3. This result were similar to Effat<sup>22</sup> and El-Kholy<sup>42</sup>. Hereupon, the inhibitory effect upon coliform organisms in treatment cheese could be attributed to the attained high acidity of acidic metabolites end products such

as (lactic-acetic acids) and the reuterin bacteriocin produced by *L. reuteri* which might prevent the growth of coliform particularly in low salt<sup>34,44</sup>.

Figure 4 showed that mould and yeast were presented in all control and cheese treatments when fresh and during cold storage period. During storage period mould and yeast in cheese treatments were insignificantly gradually decreased after 7 days of storage period ( $p > 0.05$ ) and significantly ( $p < 0.05$ ) reached their minimum level at the end of the storage period if compared with control, in which the counts significant increased rapidly ( $p < 0.05$ ).

The mould and yeast were undetected in T<sub>1</sub> (3% mushroom) at the end of the cold storage period. Our findings were in accordance with El-Gendy and Marth<sup>45</sup> who stated that the presence of or addition of *Lactococcus lactis* subsp., *lactis* to cultures of spergilli retarded mould growth for up to 2 weeks. It is also partially in agreement with Wiseman and Marth<sup>46</sup> and Coallier-Ascah and Idziak<sup>47</sup>. Moreover, El-Ziney and Debevere<sup>34</sup> reported that bacteriocin which produced by *Lactobacillus reuteri* exhibits an inhibitory activity against abroad range of microorganisms including yeast and fungi, also Effat<sup>22</sup> found that the most promising cultures having abroad spectrum of antifungal activity was *Lactobacillus reuteri*. The count of *L. reuteri* in cheese treatments was significantly increased ( $p < 0.05$ ) during cold storage period if compared with control, followed by slight decrease at the end of storage period (Fig. 5). These results were similar to which reported by Effat<sup>22</sup>. The highest counts of *L. reuteri* were recorded for functional Tallaga cheese made with 3, 5 and 10% mushroom. This might revealed the stimulation influence of the added mushroom on the growth and attitude of *L. reuteri*. Du Toit *et al.*<sup>48</sup> reported that the survival of *L. reuteri* could be attributed to the ability of *L. reuteri* to grow at low pH and may thus be regarded as acid tolerant.

At the same point of view, El-Kholy<sup>42</sup> reported that Gargir Juice extract exerted stimulatory effect on lactic acid bacteria. However, the results of this study were in the line of Casas *et al.*<sup>12</sup> who reported that *L. reuteri* can survive in various dairy products well for 7-30 days. As for lactic acid bacteria count (starter cultures) (LAB), results in Fig. 6 showed that the addition of 3, 5 and 10% mushroom to cheese milk insignificantly ( $p > 0.05$ ) increased lactic acid bacteria number in fresh functional Tallaga cheese if compared with control and this increase was much higher after 7 days of cold storage period. During storage, lactic acid bacteria counts gradually decreased reaching to a minimum level at the end of cold storage period. Our findings were agreed with those obtained by Mehanna *et al.*<sup>49</sup> and Effat<sup>22</sup>.

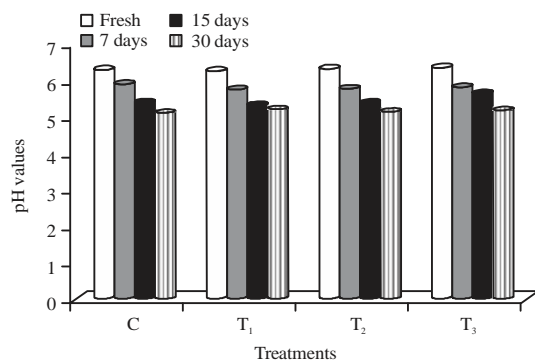


Fig. 7: Changes in pH values of Tallaga cheese fortified with 3, 5 and 10% of mushroom during 30 days of cold storage period, C: Control, T<sub>1</sub>: 3% of mushroom, T<sub>2</sub>: 5% of mushroom and T<sub>3</sub>: 10% mushroom

Table 2: Chemical composition of fresh Tallaga cheese samples

Chemical composition (%)	Cheese treatments			
	Control	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>
TS	33.52 <sup>a</sup>	36.49 <sup>b</sup>	38.58 <sup>c</sup>	40.88 <sup>d</sup>
TP	12.65 <sup>a</sup>	13.90 <sup>b</sup>	14.89 <sup>c</sup>	16.08 <sup>d</sup>
Protein/DM	37.73 <sup>a</sup>	38.09 <sup>a</sup>	38.60 <sup>b</sup>	39.33 <sup>b</sup>
Fat	13.12 <sup>a</sup>	13.52 <sup>b</sup>	13.71 <sup>b</sup>	13.75 <sup>b</sup>
Ash	3.90 <sup>a</sup>	4.87 <sup>b</sup>	5.99 <sup>c</sup>	6.80 <sup>d</sup>
Crud fiber	ND	0.37 <sup>a</sup>	0.56 <sup>b</sup>	0.87 <sup>c</sup>
Carbohydrate	3.85 <sup>a</sup>	3.83 <sup>a</sup>	3.43 <sup>a</sup>	3.38 <sup>a</sup>

ND: Not detected, <sup>a-d</sup>Means in the same row with various superscripts are significantly difference ( $p \leq 0.05$ ), TS: Total solids, TP: Total protein

In general, this study on Tallaga cheese made with addition of mushroom containing *Lactobacillus reuteri* and lactic acid bacteria demonstrated that these microorganisms can be introduced with mushroom into fresh Tallaga cheese and till 1 month of cold storage period. At that time numbers well still above the recommended threshold for probiotic effect. Therefore, Tallaga cheese with added mushroom could be used as good source for delivering probiotic as well as lactic acid bacteria to the consumers.

**Chemical analysis:** All fresh samples were analyzed for TS, TP, fat, ash, crud fiber and carbohydrate contents (Table 2). It was observed that addition of mushroom with increasing percentage had significantly ( $p \leq 0.05$ ) increased TS, TP, crude fiber and total ash. Total solids were (33.52, 36.49, 38.58 and 40.88%) for control, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> cheese samples respectively. Many literatures explained that mushroom is a valuable material rich with a considerable amount of protein. Also, the development stage of the mushrooms is a significant factor affecting the protein content. In addition, the type of mushroom, the part sampled and the location also affect protein content<sup>50</sup>. Considering of being a source of protein,

mushroom also has a reasonable amount of minerals including Na, K, Ca, Fe, Zn, Cu, P, Cd and Pb, these results are in accordance with Guillamon *et al.*<sup>51</sup>. While, there were observed that no significant differences in fat ratios with increasing ratios of mushroom between all treatment cheese samples and a little difference compared to control samples (13.12, 13.52, 13.71 and 13.75) for control, T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub> respectively. This is due to mushroom is a poor source of fat content.

While, Fig. 7 presented pH values of fresh and stored Tallaga cheese compared to control samples. The values of pH were shown a decrease trend as well as progress in cold storage period in all samples. It was observed that no significant differences ( $p \geq 0.05$ ) between fresh and 7 days of control and treated samples, while after 15 and 30 days pH values significantly ( $p \leq 0.05$ ) decreased compared to control samples. The decrease in pH values might be cause due to the impact of starter cultures or increase in lactic acid levels and also the effect of degradation of protein in samples. Also, it could be happened because of the lactic acid bacteria counts during storage period. This finding was in accordance with Dhuol and Hamid<sup>52</sup> who reported that pH values were decreased during storage period in white soft cheese. On the same trend, Sert *et al.*<sup>53</sup> found that the reduction in pH levels might be expected with activity of lactococci and lactobacilli (the main makers of lactic acid), which were the victorious microbial group at the beginning of the ripening period in Turkish Kasar cheese.

Diacetyl is natural byproducts of fermentation. It is a vicinal diketone with the molecular formula C<sub>4</sub>H<sub>6</sub>O<sub>2</sub>. Diacetyl occurs naturally in alcoholic beverages and is added to some foods to impart a buttery flavor. Diacetyl values in all Tallaga cheese samples were observed in Fig. 8, when fresh and during cold storage period. Tallaga cheese samples had gained higher values of diacetyl within the first 7 days of cold storage period then took a decreasing trend at the rest of the storage period. High concentration of mushroom in Tallaga cheese had the highest diacetyl values at the 7th day in storage period then decreased their values. These results are in agreement with Taha *et al.*<sup>54</sup>, Salem *et al.*<sup>55</sup> and Zaky *et al.*<sup>56</sup>. They all agreed that diacetyl increased in the first 7 or 10 days in cold storage then decreased in the rest storage period.

Acetaldehyde is an organic chemical compound with the formula CH<sub>3</sub>CHO, sometimes abbreviated by chemists as MeCHO. It is one of the most important aldehydes, occurring widely in nature and being produced on a large scale industrially. It is a potent volatile flavor compound that can be found in many dairy products such as cheese and yoghurt. Acetaldehyde values were shown in Fig. 9, which represented



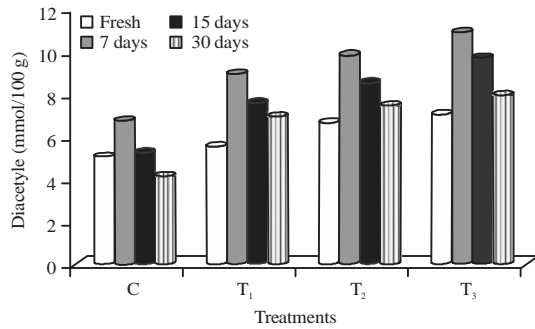


Fig. 8: Diacetyl contents of Tallaga cheese fortified with 3, 5 and 10% of mushroom during 30 days of cold storage period, C: Control, T<sub>1</sub>: 3% of mushroom, T<sub>2</sub>: 5% of mushroom and T<sub>3</sub>: 10% mushroom

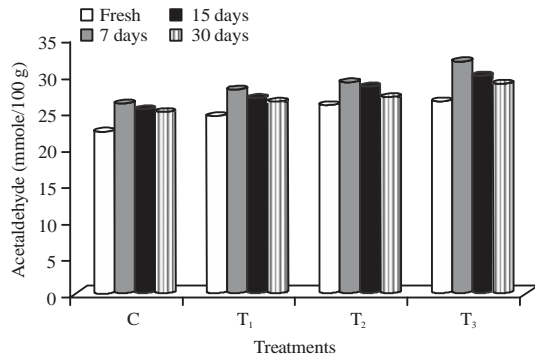


Fig. 9: Acetaldehyde contents of Tallaga cheese fortified with 3, 5 and 10% of mushroom during 30 days of cold storage period, C: Control, T<sub>1</sub>: 3% of mushroom, T<sub>2</sub>: 5% of mushroom and T<sub>3</sub>: 10% mushroom

an increasing regard in acetaldehyde values in all Tallaga cheese samples and reached the maximum values in the 7th day of storage period. The values decreased gradually after 7th day of storage period. As well as increased mushroom concentration in Tallaga cheese the acetaldehyde values increased gradually till 7th day then decreased during storage period. These data were as close as it is found in Al-Otaibi and El-Demerdash<sup>57</sup> and Pourahmad *et al.*<sup>58</sup>.

Variations in the concentration of the measured compounds (acetaldehyde and diacetyl) were mainly a result of the relevant catabolic pathways of lactose breakdown. It is important to consider that not all of the pathways are common to all of the microorganisms involved in the fermentation of milk<sup>59</sup>.

**Organoleptic properties:** Organoleptic properties are one of the most important factors in desirable product for the consumer. New product will only succeed when the consumer agree with it and vice versa. Flavor development of cheese is

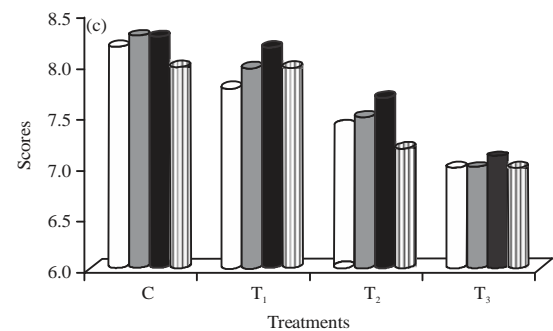
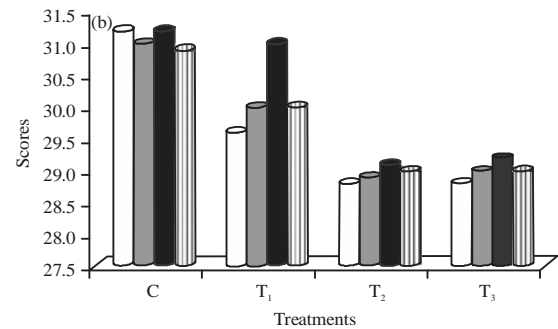
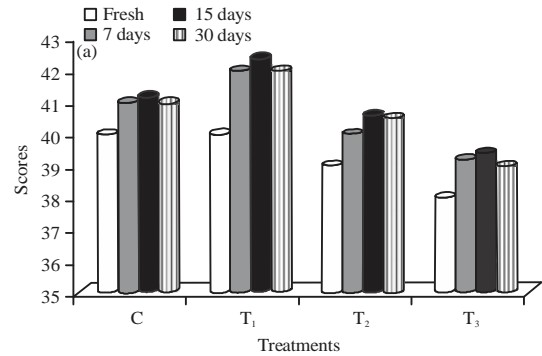


Fig. 10(a-c): Organoleptic properties of Tallaga cheese fortified with 3, 5 and 10% of mushroom during 30 days of cold storage period, (a) Flavor, (b) Body and texture and (c) Appearance, C: Control, T<sub>1</sub>: 3% of mushroom, T<sub>2</sub>: 5% of mushroom, T<sub>3</sub>: 10% of mushroom

conditional upon various biochemical changes of the product including lipolysis, proteolysis, lactose fermentation and formation of volatile compounds. So, aroma compounds of different cheese varieties occur and cheese has special characteristics during maturation according to milk type, rennet properties, manufacturing process and ripening conditions and additives such as spices (mint, nigella sativa and chili pepper) and different herbs<sup>60</sup>. So, Fig. 10 illustrated the acceptability of our new product Tallaga cheese to the panelist according to some parameters such as flavor, body, texture and appearance.

However, flavor parameter there were significant differences ( $p < 0.05$ ) between control compared to treatments

samples when fresh or after cold storage period. About 3% mushroom (T<sub>1</sub>) was gained the highest score for flavor at fresh and during cold storage period. While, cheese with 10% mushroom (T<sub>3</sub>) had gained the lowest score. During the interval storage periods there was an improvement for flavor in all cheese treatments in order after 2 weeks. This flavor could be attributing to the sensory characteristics of mushrooms, including their unique aroma and taste. Also, Maga<sup>61</sup>, Chiron and Michelot<sup>62</sup> and Tsai *et al.*<sup>63</sup> illustrated that mushrooms contain a wide range of molecules responsible for the fungal flavor comprising of volatile compounds and unsaturated fatty acids as well as amino acids.

In respect to the average score points of body and texture parameter of all fresh Tallaga cheese samples they were closed each other. The average score became slightly high after 2 weeks of storage period. By the end of storage period (30 days) all cheese samples had less or the same score. Data had cleared that body and texture were increased during 2 weeks of storage period then started to decrease till the end of experiment. Although appearance of control samples had higher score than all treatment samples, the appearance parameter started to decrease all over the interval storage period. From all previous results, it is found that Tallaga cheese with 3% mushroom (T<sub>1</sub>) was gained the highest scores for each of sensory parameters comparable to control cheese sample.

## CONCLUSION

For consumers who prefer unique and desirable products; a remarkable Tallaga cheese fortified with probiotic bacteria and mushroom was presented. Consequently, the chemical, microbiological and sensory evaluation indicated that Tallaga cheese with 3% of mushroom had the highest scores compared to other treatments.

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