

Potential Inhibitory Effect of Probiotic *Lactococcus lactis* and *Lactobacillus acidophilus* on *Helicobacter pylori* in Traditional Egyptian White Soft Cheese: *In vitro* Study



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Abstract

Several investigations have considered *Helicobacter pylori* as a foodborne pathogen and have detected in different milk products such as Kariesh and Talaga cheeses, which are common dairy products in the Egyptian market. Traditional specific culture media and methods were followed up in studying the activity and vitality of *Lactobacillus acidophilus* and or *Lactococcus lactis* in Kariesh and Talaga cheeses contaminated with *H. pylori* and their inhibitory effect, as Inhibition Ratio % (InhR %), on the bacterial pathogen. *H. pylori* survived for one month in Kariesh cheese samples whether they were manufactured with co-culture of *Lac. lactis* and *Lb. acidophilus* or *Lac. lactis* solely, during 4 weeks cold storage. In addition, *H. pylori* survived in Tallaga cheese samples containing *Lb. acidophilus* with gradual decrease during the first three weeks cold storage and complete inhibition (InhR %) for the pathogen in the 4th week. Physico-chemical changes of the cheese samples, such as pH, fat, moisture and salt % were assessed and showed no remarkable or significant effects on either *H. pylori* or lactic acid bacteria during the storage period. Chemical composition or inclusion of lactic acid bacteria did not appear to have a deleterious effect to eliminate *H. pylori* in Kareish and Tallaga cheeses during the period of conservation under the investigated conditions, except in Tallaga cheese where *Lb. acidophilus* illustrated complete inhibition (100%) at the end of the experimental storage period.

Keywords: *Helicobacter pylori*, White soft cheese, *Lactobacillus acidophilus*, *Lactococcus*.

Introduction

Helicobacter pylori is a gram negative bacterium that is widespread in about 50% of the human population around the world, many studies illustrated that the rate of infection and prevalence is very high in developing countries (in some areas, >85%) compared to developed ones (about 30-40%) (Breckan *et al.*, 2016). This organism colonizes the human stomach, usually acquired in

infancy and during childhood; this colonization is not always related with the development of pathogenicity as more than 70% of the infected population remains asymptomatic (Sykora *et al.*, 2006 and Das and Paul, 2007). *H. pylori* infection was associated with pathogenesis of many gastrointestinal diseases such as diarrhea, chronic abdominal pain, peptic ulcers and gastric adenocarcinoma (Tokudome *et al.*, 2006 and Kuo *et*

al., 2013). Many authors have considered *H. pylori* as a foodborne pathogen since several studies have detected the survival and presence of this microorganism in different food-stuffs. El Dairouty *et al.* (2016) and Hamadaa *et al.* (2018) isolated *H. pylori* from foods of animal origin while it was isolated by Saeidi and Sheikhshahrokh (2016) and El Gamal *et al.* (2012) from cow milk, sheep, goat and camel milks. Furthermore, Saad and El-Prince (2004) isolated *Helicobacter* species from raw marketable milk, Kareish cheese and cooking butter samples from Assiut province. Milk is an important nutritious food for the human in different ages; limited studies are available concerning the occurrence of *H. pylori* in dairy products other than milk (Mousavi *et al.*, 2014). Jiang and Doyle (2002) observed that fresh milk not likely to contain *H. pylori* but it was contaminated because of inadequate hygiene, the bacteria might survive long enough to cause infection. Cheese is dairy product supplemented with various healthy additives including probiotic cultures, and Lactic Acid Bacteria (LAB) is suitable transfer medium for the probiotic microorganisms to the human gastrointestinal tract (Karimi *et al.*, 2011). Probiotic bacteria are living microorganisms that, when present in sufficient amounts, introduce benefits to host health, furthermore, the probiotics and their metabolic products are correlated to reduction of allergies, cancer, hepatic disease, *H. pylori* and urinary tract infections (Ejtahed *et al.*, 2011; Lollo *et al.*, 2013 and Nabavi *et al.*, 2014). Lactic acid bacteria are the most common probiotic agents being

used in dairy industry; therefore interest has been focused on the incorporation of LAB into cultured dairy products such as cheese and fermented milk which are known to be essentially fermented by LAB (Boylston *et al.*, 2004). The addition of probiotics to soft cheese made the product more acceptable in sensory properties enhanced the shelf life and can produce cheese with high quality and health aspects (Effat *et al.*, 2018). The ability to produce antimicrobial factors is considered an essential characteristic of probiotic microorganisms; generally their effect on the activities of foodborne (Madureira *et al.*, 2011). Foodborne disease is a severe problem worldwide, different strategies are required to control or kill pathogens and conserve food (Claude *et al.*, 2016), therefore this study was planned to examine the potential inhibitory effect of *Lb. acidophilus* and or *Lac. lactis* as probiotics on the growth and survival of *H. pylori* in two Egyptian white soft cheeses (Kareish and Tallaga) during one month of cold storage. Also, some physico-chemical properties for the cheese as affecting factors were taken in concern throughout this study.

Materials and Methods

Reference strains of bacteria.

Helicobacter pylori as a test strain was isolated from milk and milk products at Dairy Department, National Research Center; Dokki, Egypt. Isolation and identification was carried out according to Quaglia *et al.* (2007) and El Gamal *et al.* (2012). The isolated strain was sub-cultured and maintained using Brain Heart Infusion Agar (BHIA) until

use. Commercial starter cultures of *Lb. acidophilus* (La-5) and *Lac. lactis* subsp. *Lactis* (CH-1) were obtained from Chr. Hansen's Lab., Denmark. They were cultivated and maintained on DeMan Rogosa Sharpe (MRS) and M17 respectively, and incubated at 30-37°C for 24 h until sufficient growth (10^6 CFU/g). The cultures were diluted in saline solutions to an appropriate inocula size and were ready for inoculation during cheese preparation.

Manufacture and samples of Egyptian white soft cheese.

Two kinds of Egyptian white soft cheese (Kareish and Tallaga) were manufactured in Dairy Department, National Research Center; Dokki, Egypt using probiotic bacterial cultures and assessed for contamination with the *H. pylori* isolate.

1- Kareish cheese inoculation.

Kareish cheese was made according to the method of Effat *et al.* (2001). as follow: skimmed cow's milk was pasteurized at 72 °C for 16 sec., cooled to 40 °C then after 3% NaCl was added. The milk was divided into two portions (1L /each) and were treated as follow:

The first portion: was inoculated with *H. pylori* $\sim 10^6$ cfu/ml and mono Lactic Culture (mono-LC) of *Lb. lactis* in ratio 2.0% (cheese starter) and served as control sample.

The second portion: was contaminated with *H. pylori* $\sim 10^6$ cfu/ml, and inoculated with di- Lactic Culture (di-LC) of *Lb. acidophilus* $\sim 10^7$ cfu/ml and *Lac. lactis* in ratio 2.0% and served as treated sample.

2- Tallaga cheese inoculation.

Tallaga cheese was prepared following the producer of Abd El Sa-

lam *et al.* (1981), in which cow milk was heat treated to 72 °C for 16 sec., cooled to 40 °C then 4% NaCl was added. The milk was divided into two portions (1L /each) and was treated as follow:

The first portion: was contaminated with *H. pylori* $\sim 10^6$ cfu/ml, served as control sample.

The second portion: was contaminated with *H. pylori* $\sim 10^6$ cfu/ml and inoculated with Mono- Lactic culture of *Lb. acidophilus* $\sim 10^7$ cfu/ml, was served as treated sample. After milk inoculation and fermentation calf rennet (1g/100L) was added and left to complete coagulation, then kept in refrigerator with care to avoid post contamination.

Cheese samples preparation.

Samples of Kareish and Tallaga cheeses were aseptically cut into approximately 5x5 cm pieces and placed in sterilized white capped plastic cups and kept in refrigerator at 4 °C for further estimations. Meanwhile, amount of 25 g of each sample was smashed for 2 min, with 225ml of enrichment broth (buffer peptone water) using sterile mortar, Samples were subsequently diluted (1:10) using sterile saline water. All samples were subjected to bacteriological and chemical assessment.

Bacteriological examination.

Bacteriological examination was carried out to investigate the viability of the incorporated pathogen (*H. pylori*) as affected by the probiotic LAB (*Lb. acidophilus* and *Lac. lactis*) in cheese samples directly after manufactured (fresh samples) and at four different cold storage periods: 1, 2, 3, and 4 weeks. For viability assessment each organism was recov-

ered and enumerated onto its selective agar media. Enumeration of *H. pylori* was carried out on BHIA plates supplemented with 7% sterile horse serum and *H. pylori* selective supplement (SR0147E), as recommended by Quaglia *et al.* (2007). Plates were incubated for 5-10 days at 37°C under micro-aerophilic condition. Lactic acid bacteria, *Lb. lactis* were enumerated on M17 agar plates and incubated at 30 °C for 24h (Laetitia Gemelas *et al.*, 2013). While, *Lb. acidophilus* were enumerated using MRS agar medium and incubated anaerobically using anaerobic gas pack Jar at 37 °C for 48 h (Collins, 1978). The viable count (CFU/g) for each strain was determined by considering the mean plates count. Experiments were performed in triplicate for each treatment to validate the results through statistical analysis.

InhR % of *H. pylori* in the investigated cheese as affected by addition of LAB.

The InhR % of the pathogenic strain (PS) was evaluated for Tallaga and Kareish cheeses due to addition of *Lb. acidophilus* and *Lac. lactis* as Mono- and Di-LC. Estimation was done according to the following equations as reported by Amal *et al.* (2016) and Okda *et al.* (2018).

InhR % for Mono- lactic culture.

$$\text{Log cfu g}^{-1} \text{ C PS} - \text{log cfu g}^{-1} \text{ PS}$$
$$\text{in Mono-L C X 100) / log cfu g}^{-1} \text{ C PS}$$

InhR % for Di- lactic cultures.

$$\text{Log cfu g}^{-1} \text{ CPS} - \text{log cfu g}^{-1} \text{ PS}$$
$$\text{in Di- LC X 100) / log cfu g}^{-1} \text{ C PS}$$

-CPS = Control Pathogenic Strain (count of *H. pylori* in Tallaga cheese with no lactic cultures).

-PS in mono-LC = Pathogenic Strain count in cheese samples treated with *Lb. acidophilus* or *Lac. lactis* solely.

-PS in di- LC = Pathogenic Strain count in cheese samples treated with *Lb. acidophilus* and *Lac. lactis*.

Physico-chemical examinations.

Cheese samples were analyzed to determine out some of the physico-chemical parameters (pH value, moisture, fat, salt contents) at the same time intervals as described for bacteriological analysis. The pH value of the cheese was determined at room temperature using digital pH meter (Hanna, Germany) equipped with a combined electrode, temperature meter Model 3505 Jenway, England was used for temperature detection at zero time, Moisture was estimated according to the method described by Ling (1963). Cheese samples were analyzed for fat contents according to AOAC (2012) methods and salt was estimated as NaCl according to Richardson (1985).

Statistical Analysis:

Statistical analyses were performed using the GLM procedure with SAS (2004) software. Duncan's multiple comparison procedure was used to compare the means. A probability to $P \leq 0.05$ was used establish the statistical significance.

Results and Discussion

Viability and vitality of *H. pylori* in Kareish cheese with mono and di-LAB cultures.

At recent days, there has been an increasing concern for incorporation of probiotics into fermented milk products to reduce spoilage and inhibit pathogenic bacteria as a safe way of preservation (Claude *et al.*, 2016). In the present study survival of

H. pylori in Kareish cheese which contained LAB culture of *Lac. lactis* during the conservation period (one month) in the refrigerator recorded very slight decrease not significant (Fig. 1). While *Lac. lactis* and Total Bacterial Count (TBC) showed a significant increase after the second week of conservation. On the other hand, pathogen viability in Kareish samples, containing *Lac. lactis* and *Lb. acidophilus*, remained until the third week of storage and showed remarkable decline at the fourth week (Fig. 2). In accordance, Lionetti *et al.* (2010) reported that different *Lactobacillus* strains including *Lb. acidophilus* can inhibit the growth of *H. pylori* *in vitro* and *in vivo*. Zeinab *et al.* (2011) also recorded a rapid decline in initial numbers and survival of *H. pylori* in yoghurt supplemented with *Lb. acidophilus* and no viable count found after 3 days. Different organic acids (formic, acetic, lactic, propionic, benzoic and free fatty acids) are produced as metabolic by-products of probiotic bacteria (Gummalla and Broadbent, 2001 and Magnusson, 2003) which can provided various modes for the inhibition of pathogenic and spoilage organisms. Several authors have linked the inhibitory effect of probiotics by the weakness of the acid produced since

the un-dissociated forms of weak organic acids can diffuse easily through the pathogenic cell membrane and dissociate inside the cells. The H^+ ions which released during the dissociation cause disorder of the electrochemical proton gradient, resulting in an eventual death of the pathogen (Piard and Desmazeaud, 1991 and Pieter *et al.*, 2016). It was concluded also that both probiotic cultures added and TBC were present in high levels during the entire storage period with remarkable increase in the second and third weeks (Fig. 2). In agreement, El-Kholy *et al.* (2016) and Yaser *et al.* (2019) recorded increasing in total bacterial counts and the *lactobacilli* strains (*Lb. casei*; *Lb. johnsonii* and *Lb. acidophilus*) added to white soft cheese up to 21 days followed by gradual decrease up to the end of storage. Azizkhani and Tooryan (2016) illustrated that the increase in probiotics count during the first 7 days of storage was related to the reduction in pH, on the other hand Shah (2000) and El-Abd *et al.* (2003) assumed that the ultimate reduction in bacterial counts might be attributed to the accumulation of organic acids as a result of growth and fermentation which control rate of bacterial growth.

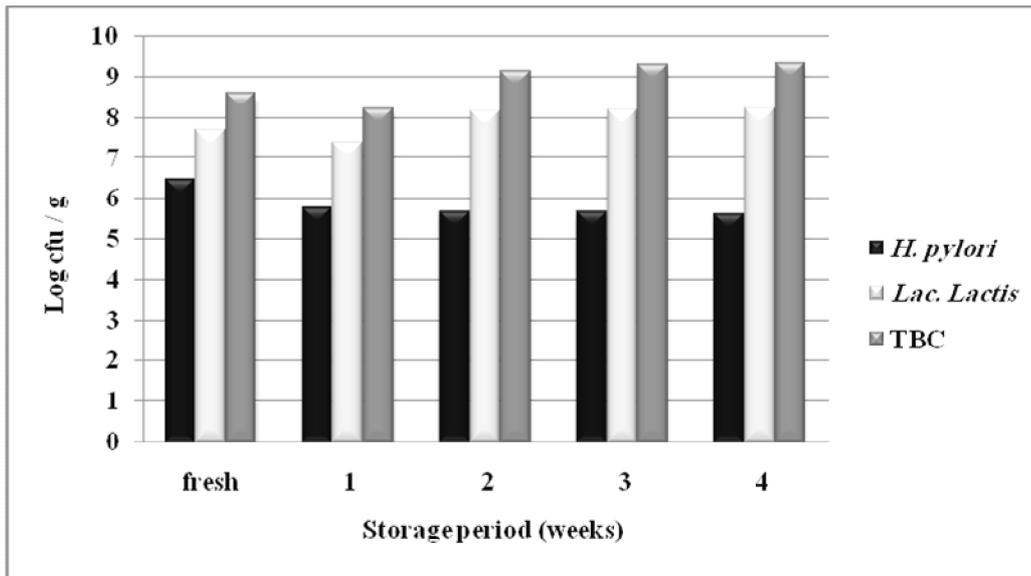


Fig. 1. Viability of *H. pylori* in Kareish cheese with probiotic *Lac. lactis* during 4 weeks cold storage.

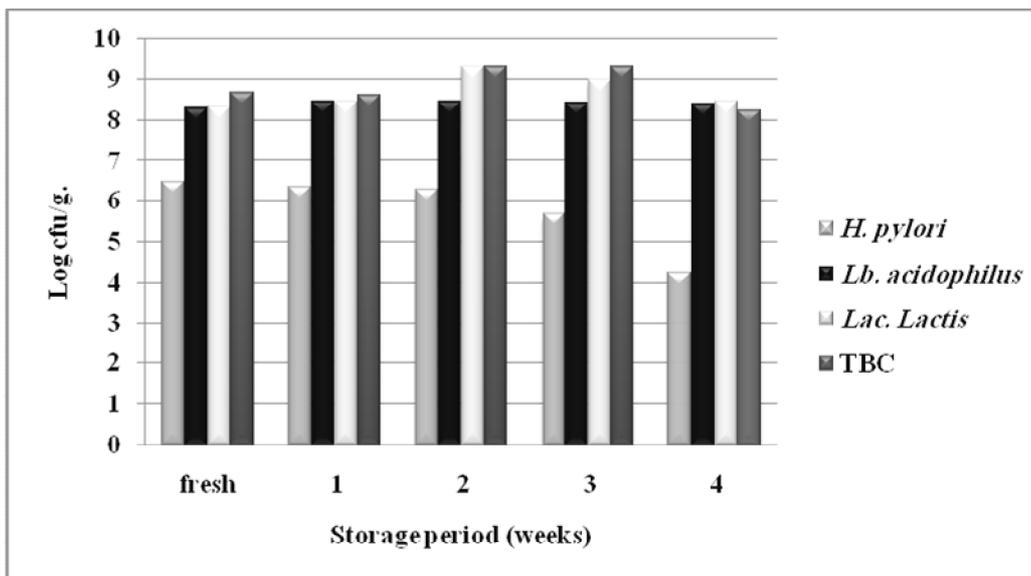


Fig. 2. Viability of *H. pylori* in Kareish cheese with probiotic *Lb. acidophilus* and *Lac. lactis* as di-culture during 4 weeks cold storage.

Viability and vitality of *H. pylori* in Tallaga cheese with mono- LAB culture.

Tallaga cheese, which is not supported by any LAB bacteria (control), showed a gradual decrease in *H. pylori* count associated with gradual increase in TBC during cold cheese preservation (Fig. 3). While Tallaga

cheese supported by *Lb. acidophilus* as mono-LAB culture (Fig. 4), showed remarkable decrease of *H. pylori* from 10^6 to 10^3 cfu/g during the first 3 weeks of cold storage then no viable count was detected at the end of storage period signifying complete inhibitory effect of *Lb. acidophilus* strain. Results are in line with Yaser

et al. (2019) who found that *Lb. acidophilus* decreased spoilage in low salt soft cheese with greater inhibitory effect than *Lac. casei*. Collado *et al.* (2005) illustrated that *Lb. acidophilus* applies its antagonistic effect against *H. pylori* during the production of a heat stable peptides that exert antimicrobial effect other than lactic acid. While Le Moal *et al.* (2013) suggested unusual mechanism by probiotic lactobacilli against pathogenic *H. pylori* in which the lactic acid produced making morphological changes of the pathogen and replacing the helical cells by “c”-shaped or coccoid cells which lead to irreversible inhibition of the swimming motility. In respect to viability of the bacteria added (*Lac. acidophi-*

lus and *Lac. lactis*) as well as TBC the current results illustrated a gradual increase in consistent manner in all cheese samples (Kareish and Talaga) during the first weeks and start to decrease by progressing storage time. Results are in consistent with El-kholy (2007) who observed an increased total viable count as well as *Lac. casei* added to a maximum during the first two weeks storage of manufactured Domiati cheese, and then decreased slightly thereafter. Donkor *et al.* (2007) explained that extending storage time play an important role in the overall proteolytic activity, and subsequent increase in the amount of amino acids produced which may cause a higher growth rate of probiotic bacteria.

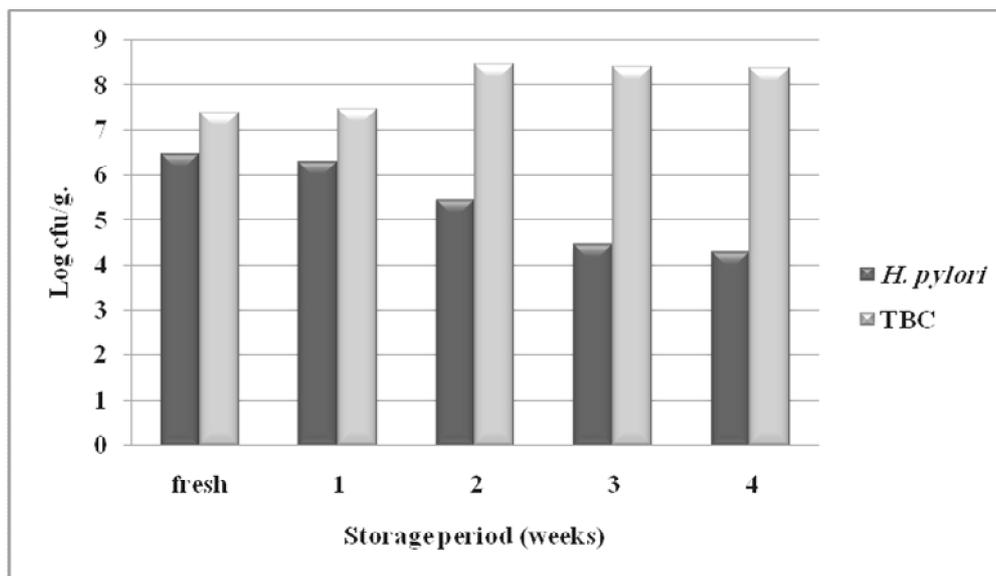


Fig. 3. Viability of *H. pylori* in Tallaga cheese (free from LAB) during 4 weeks cold storage.

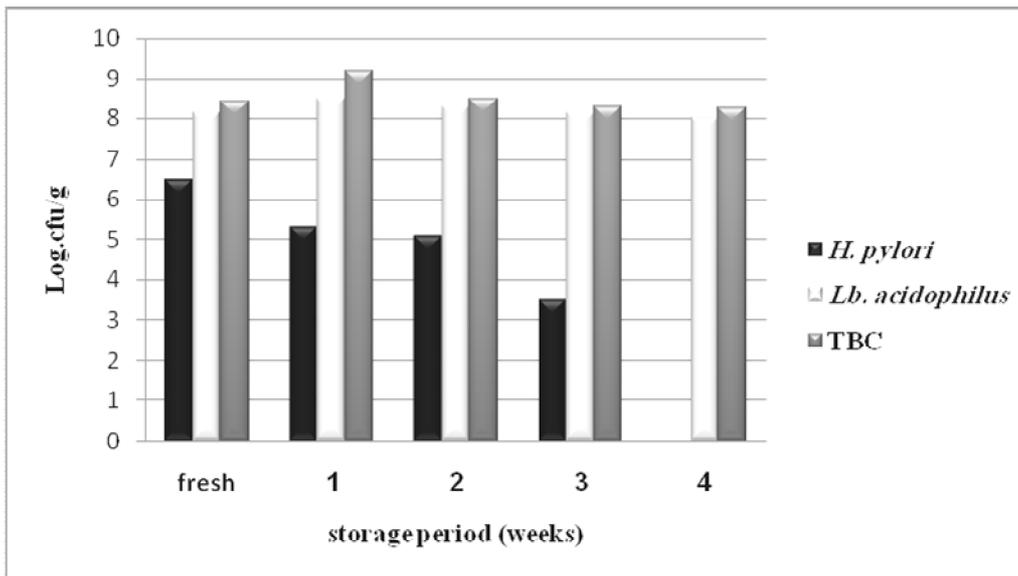


Fig. 4: Viability of *H. pylori* in Tallaga cheese with *Lb. acidophilus* during 4 weeks cold storage.

Inhibition ratio % of *H. pylori* in the studied cheeses as a result of LAB addition.

Results illustrated no probable effect for *Lac. lactis* and *Lb. acidophilus* either as mono- or di-LAB cultures against *H. pylori* in Kareish cheese during the 4 weeks cold storage, since negative inhibition response was recorded. However, good response for *Lb. acidophilus* against *H. pylori* in the contaminated Tallaga cheese as complete inhibition (100%) was reached by the end of the storage period (Table 1). Zeinab *et al.* (2011)

demonstrated that the decline in numbers of *H. pylori* is correlated with a reduction in pH values as a result of probiotic strains proliferation, which might be due to convert residual lactose in cheese to short chain free fatty acid as metabolic end product (Magdoub *et al.*, 2005). Or increase in lactic acid levels and degradation of protein in samples which might be expected with progression activity of probiotics (Dhuol and Hamid, 2013 and El-Kholy *et al.*, 2016).

Table 1. Inhibition ratio % of *H. pylori* in cheese samples due to the presence of LAB, in mono and di- cultures during 4 weeks cold storage.

Treatments	Tallaga cheese with <i>Lb. acidophilus</i> (control)	Kareish cheese with <i>Lac. lactis</i>	Kareish cheese with <i>Lac. lactis</i> and <i>Lb. acidophilus</i>
Fresh	0	0	0
1 week	+15.9	+8.41	-0.6
2 weeks	+7.3	-4.0	-14.6
3 weeks	+21.7	-27.3	-27.3
4 weeks	+100	-30.7	+1.63
+ indicates positive response - indicates negative response			

Physico-chemical analysis of studied cheese samples.

Changes in some physico-chemical properties of cheese samples during cold storage were studied and illustrated similar pattern of development for the two types of cheeses. Reduced values of pH and moisture % were detected as well as progress in cold storage time in all cheese samples, while fat, and salt % development showed an increasing trend that was in consistent with Ezzat (1990) and El-Zayat and Osman (2001). This alteration in cheese samples during the four weeks cold storage showed no significant effects on survival of *H. pylori* or lactic acid bacteria studied (Tables 2 and 3). This is in spite of the lower pH value in Kareish cheese (3.9) than in Talaga cheese (4.7) and the higher percentages of fat (12.8 %) and salt contents (5.1 %) in the latter, at the end of cold storage. In agreement, increasing acidity was recorded by Flimelova *et al.* (2013) which resulted mainly from activity of *Lb. acidophilus* as a part of the culture this was also confirmed by many published reports (Dhuol and Hamid, 2013 and El-Kholy *et al.*, 2016). On the other hand, the current results did

not agree with, Peroti *et al.* (2014); Yasser *et al.* (2019) and Zeinab *et al.* (2011), who demonstrated that *H. pylori* reduction was associated with low pH values due to the proliferation of probiotic strains and the production of various organic acids as metabolic products. Scott *et al.* (2010) reported an important factor for the response of *H. pylori* to acidity, is acid acclimation, which is defined as the ability of *H. pylori* to maintain pH close to neutrality in the presence of extra bacterial acidity. In addition, Mahmoudi *et al.* (2012) observed that the decrease in moisture content is clearly associated with increase in salt amount. The current results were in consistent with Souza and Saad (2009) who recorded significant reduction in moisture content of cheese samples and increasing percent salt as preserving period proceeded. Interestingly, they assumed that during acidification the concentration of hydrogen ions increases, the repulsive forces decrease, and the casein start to aggregate the considerable removal of whey during storage resulted in a significant decrease in moisture of cheeses. These results are in agreement with those obtained by Kebary *et al.* (2015).

Table 2. Physico-chemical analysis of Kareish cheese during 4 weeks cold storage.

Storage time	pH		Fat (%)		Moisture (%)		Salt (%)	
	control	treated	control	Treated	control	treated	control	treated
Fresh	4.53Aa ±0.033	4.7Aa ±0.001	1.8Ca ±0.007	1.8Ba ±0.001	73.33Aa ±0.88	73.6Aa ±0.577	3.1Ca ±0.01	3.0Ca ±0.057
1 week	4.3Aa ±0.05	4.43Aa ±0.088	2.0Ba ±0.05	1.8Ba ±0.09	71.33Aa ±0.88	71.5Aa ±0.60	3.5Ba ±0.15	3.5Ba ±0.057
2 weeks	3.9Aa ±0.057	4.33Aa ±0.057	2.01Ba ±0.044	1.9Ba ±0.057	68.0Bb ±0.57	70.0Ba ±0.88	3.7Aa ±0.088	3.8Aa ±0.08
3 weeks	3.7Bb ±0.044	4.3Aa ±0.044	2.1Ba ±0.057	1.95Bb ±0.0166	66.33Ba ±0.33	65.5Cb ±0.577	3.9Aa ±0.1	3.9Aa ±0.057
4 weeks	3.55Bb ±0.033	3.9Ba ±0.04	2.3Aa ±0.057	2.0Ab ±0.06	60.5Ca ±0.28	60.0Db ±0.57	4.1Aa ±0.057	4.0Aa ±0.057

-Means with the same capital letters between columns and small letters between rows are not significantly different ($p \leq 0.05$).

-Control samples contain *Lac. lactis*, Treated samples contain *Lb. acidophilus* and *Lac. lactis*.

Table 3. Physico-chemical analysis of Tallaga cheese during 4 weeks cold storage.

Storage time	pH		Fat (%)		Moisture (%)		Salt (%)	
	control	treated	control	treated	control	treated	control	treated
Fresh	5.0Ab ±0.057	5.3Aa ±0.057	12.0Ba ±0.057	12.0Ba ±0.057	65.0Ab ±0.13	67.29Aa±0.05	4.0Ca ±0.033	4.0Ca ±0.001
1 week	4.6Bb ±0.017	5.1Ba ±0.029	12.3Ba ±0.17	12.0Bb ±0.11	61.0Bb ±0.57	61.98Ba±0.57	4.49Ba ±0.005	4.46Ba ±0.006
2 weeks	4.3Cb ±0.057	4.8Ca ±0.03	12.5Aa ±0.23	12.3Aa ±0.17	59.12Cb±0.75	60.8Ba ±0.39	5.0Aa ±0.008	4.98Ab ±0.02
3 weeks	4.3Cb ±0.011	4.7Ca ±0.04	12.5Aa ±0.24	12.6Aa ±0.1	55.8Db ±0.44	57.0Ca ±0.57	5.12Aa ±0.029	5.09Aa ±0.042
4 weeks	4.2Cb ±0.033	4.7Ca ±0.034	12.7Aa ±0.17	12.8Aa ±0.115	53.9Db ±0.15	56.2Ca ±0.56	5.17Aa ±0.013	5.14Aa ±0.063

-Means with the same capital letters between columns and small letters between rows are not significantly different ($p \leq 0.05$).

- Control samples no LAB, Treated samples contain *Lb. acidophilus*.

Conclusion

The *in vitro* experimental conditions of the present study indicate that the incorporation of probiotics, *Lb. acidophilus* and *Lac. lactis*, whether single or mixed cultures as well as the alteration of the physico-chemical composition were not sufficient enough to eliminate the presence of *H. pylori* in Kareish cheese. While promising results for *Lb. acidophilus* in Tallaga cheese was found, where the former strain is capable of inhibiting the *in vitro* growth of the pathogen completely at the end of the storage period. Furthermore, in terms of bacterial survival and viability it was seen that *Lb. acidophilus* and *Lac. lactis* retained high counts ($> 10^7$ cfu/g) throughout the 4 weeks cold storage demonstrating the high survival of the tested probiotics. Further studies of the inhibitory effects of *Lac. lactis* and *Lb. acidophilus* strains are important for creating different perspectives to be used in bio-control of cheese food borne pathogens. *Lb. acidophilus* in this study opens the door for additional assessments for its possible inhibitory effect against *H. pylori* in white soft cheese.

Conflict of Interest:

Authors declare that there is no conflict of interest.

Ethical approval:

This article does not contain any studies with human participants or animals performed by any of the authors.

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دراسه معملية لإختبار قدرة بكتريا حمض اللاكتيك لآكتوباسيلس لآكتيس و لآكتوباسيلس أسيدوفيلس علي تثبيط نمو بكتريا هاليكوبآكتر بايلوري في الجبنه البيضاء

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المخلص

كثير من الأبحاث تعتبر الهاليكوبآكتر من الكائنات التي تسبب فساد الأغذية و قد ثبت وجوده في كثير من منتجات الألبان مثل الجبن القريش و الثلآجه والتي تعتبر من المنتجات الشائعة في مصر. وقد أستخدمت الطرق و البيئات التقليدية لمتابعة و دراسة نمو و تأثير بكتريا اللاكتوباسيلس أسيدوفيلس و لآكتوباسيلس لآكتيس كمزرعه مزدوجه أو لآكتوباسيلس لآكتيس كمزرعه منفردة علي الجبن القريش و الثلآجه الملوثة ببكتريا الهاليكوبآكتر بايلوري. وقد لوحظ أن البكتريا الممرضه أستطاعت النمو لمدة شهر في عينات الجبن القريش المصنعه في وجود مزرعه مزدوجه أو منفردة من سلالات بكتريا حمض اللاكتيك تحت ظروف الحفظ في المبرد مع انخفاض تدريجي غير معنوي. و من ناحية أخرى أستطاعت البكتريا الممرضه النمو في عينات الجبن الثلآجه المحتويه علي مزرعه منفردة من بكتريا اللاكتوباسيلس أسيدوفيلس فقط مع انخفاض تدريجي في النمو خلال الثلاث أسابيع الأولى و إنعدام النمو في الأسبوع الرابع من فترة الحفظ. كذلك تم دراسة بعض التغيرات الفيزيائية و الكيميائية لعينات الجبنه تحت الأختبار مثل pH , نسبة الدهن , الرطوبة و الأملاح في خلال الأربعة أسابيع من الحفظ في المبرد وقد لوحظ عدم تأثير للتغيرات التي حدثت في عينات الجبن علي نمو و نشاط سواء بكتريا حمض اللاكتيك أو البكتريا الممرضه. و علي ذلك فقد تبين عدم وجود تأثير لبكتريا حمض اللاكتيك المستخدمه علي البكتريا الممرضه في عينات الجبن القريش، في حين أثرت بكتريا اللاكتوباسيلس أسيدوفيلس علي البكتريا الممرضه في عينات الجبن الثلآجه وأدت الي تثبيط كامل للبكتريا الممرضه في نهاية فترة الحفظ.