Open Veterinary Journal, (2021), Vol. 11(3): 356–363
ISSN: 2226-4485 (Print)
ISSN: 2218-6050 (Online)
Original Research
DOI: *10.5455/OVJ.2021.v11.i3.4*

Submitted: 27/02/2021 Accepted: 27/06/2021 Published: 19/07/2021

Fundamental role of *Lactobacillus plantarum* and inulin in improving safety and quality of Karish cheese

Ola W. Hegab*, Eman F. Abdel-Latif, Hamdy M.B.A. Zaki and Ashraf A. Moawad

Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt

Abstract

Background: Karish cheese manufactured traditionally from raw milk may harbor many biological health hazards.

Aim: Production of safe pasteurized Karish cheese with improved sensory characteristics using probiotics and prebiotics (synbiotic Karish cheese).

Methods: Laboratory Karish cheese was made to study the effect of *Lactobacillus plantarum* with and without inulin on cheese quality. Treatments were examined for sensory, chemical, and microbial quality, shelf life, and survival of *L. plantarum* were also monitored. The antimicrobial effect of *L. plantarum* and inulin against *Enterobacter aerogenes* in cheese was evaluated.

Results: Sensory, chemical, and microbial quality of Karish cheese supplemented with *L. plantarum* and inulin were positively affected; moreover, the shelf life was extended up to 28 days. Karish cheese contained *L. plantarum* showed the highest flavor score, while treatment contained both *L. plantarum* and inulin attained the best body and texture score. Moreover, *L. plantarum* and inulin significantly reduced *E. aerogenes* count during Karish cheese chilled storage; the reduction log reached 3.76 $\log_{10}$cfu/g on the seventh day of storage compared to control. Additionally, Inulin significantly increased the survival of *L. plantarum* throughout the storage period.

Conclusion: This study concluded that using probiotics and prebiotics in Karish cheese synergistically improved its sensory properties, safety, and hygienic quality.

Keywords: *Enterobacter aerogens*, inulin, Karish cheese, *Lactobacillus plantarum*, synbiotic.

Introduction

Recently, consuming low-fat foods includes cheeses to reduce health risks associated with high dietary fats as heart diseases, hypertension, arteriosclerosis, and obesity (Tufeanu and Tița, 2016).

Karish cheese is one of the most popular white soft skimmed milk cheeses manufactured traditionally in Egyptian villages from the raw milk. In the traditional method, raw milk is kept in earthenware pots for 24–72 hours (differs according to the season) until the cream layer is separated, then it is removed, and the remaining fermented skim milk pressed and transferred into a mat that hanged for 2–3 days to drain whey after that it is dry salted, cut into cubes, and left to drain for 2 hours before consumption (Hammam *et al*., 2020). Traditional Karish cheese produced from the raw milk under poor hygienic measures may contain several health risk contaminants (Hamad, 2015).

Industrially produced Karish cheese is manufactured using pasteurized skim milk having 0.1%–0.5% fat content relaying on the quality of the separator, with the addition of starter culture (Todaro *et al*., 2013; Allam *et al*., 2017a). Consumers usually prefer traditional Karish cheese owing to its good flavor created by native microflora of raw milk (Hegab *et al*., 2020). Additionally it contains a relatively higher fat content compared to industrial one due to different fat separation methods. It is worth mentioning that the fat acts as a flavor enhancer for cheese and gives it a characteristic texture, fat removal leading to lack of good cheese flavor and rubbery texture (Madadlou *et al*., 2005; Awad, 2016).

The challenge is to produce a safe pasteurized Karish cheese more closely resembles the conventional one in its organoleptic properties and solve fat removal problems. Several trials have been proposed to improve Karish cheese, using probiotics and prebiotics to adjust its organoleptic properties and provide it with health-promoting benefits (Ahmed *et al*., 2005; Hamad, 2015). Functional synbiotic products are considered as the modern trend in food technology that containing both probiotics and prebiotics (Angiolillo *et al*., 2014a).

Probiotics are live microorganisms that are used widely in many foodstuffs, including dairy products. They deliver many health benefits to humans when they exist in food at the time of consumption with a minimum count of 7.0 $\log_{10}$cfu/g. Probiotics contribute to the re-balance of beneficial intestinal microflora and inhibit harmful entero-pathogens that improve digestive ability and relieve constipation. In addition, probiotics decrease the blood cholesterol level, improve body immunity, and have antimutagenic and anticarcinogenic effects (Hammam and Ahmed, 2019; Ali *et al*., 2020). Probiotics also play a technological role in food, particularly dairy products; they act as

***Corresponding Author:** Ola Wagih Hegab. Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt. Email: *olawagihvet90@gmail.com*

bio-preservatives, prolong the shelf life of fresh dairy products, and improve their sensory characteristics (Angiolillo *et al*., 2014b; Allam *et al*., 2017a). Generally, cheeses are considered as better vehicles for carrying probiotics than others dairy products due to their higher pH and more solid consistency that protect probiotics in the human intestine (Modzelewska-Kapituła et al, 2007; Hussein and Shalaby, 2014).

Lactobacillus plantarum is one of the most protective and health-promoting Lactic acid bacteria (LAB). It is known to produce antimicrobial substances, e.g., plantaricin, that inhibit many pathogenic and spoilage microorganisms, for example, *Escherichia coli, Salmonella Typhimurium*, *Staphylococcus aureus, Enterobacter aerogenes*, *Proteus vulgaris*, and *Pseudomonas aeruginosa* (Dinev *et al*., 2018; Al-Gamal *et al*., 2019). Meanwhile, the *L. plantarum* DSM 20174 strain is a promising strain with many probiotic properties, which help exhibit its beneficial effects inside the human body. These probiotic properties including tolerance to acid and bile salts, resistance to many antibiotics, and production of β galactosidase enzyme that helps in lactose digestion. Moreover, it can ferment fructooligosaccharides used in synbiotic products (Cebeci and Gürakan, 2003). The same *L. plantarum* DSM 20174 strain was used in the current study.

Enterobacter aerogenes is an opportunistic bacterium that has emerged as a nosocomial pathogen as well as a spoilage microorganism. It is a member of the coliform group that characterized by acid and gas production from lactose, result in undesirable flavors, slime formation, and discoloration in dairy products; it is often isolated from raw milk and Karish cheese (Baylis, 2006; Davin-Regli and Pagès, 2015; Dinev *et al*., 2018).

Prebiotics are non-digestible carbohydrates metabolized selectively by probiotic bacteria in the large intestine, stimulating the growth of beneficial bacteria and improving gut health (Karimi *et al*., 2015). Inulin is one of the best prebiotics used in foods, especially in dairy products; it is a carbohydrate polymer consisting of fructose units, soluble dietary fiber, and generally regarded as a safe food ingredient. It is added in dairy products within an average of 2%–10%, not only to give a good viable count of probiotics during the storage period but also acts as a fat replacer and flavor enhancer (Modzelewska-Kapituła *et al*., 2007; Karimi *et al*., 2015; Junyusen *et al*., 2017).

This study aims to produce a synbiotic Karish cheese using *L. plantarum* in addition to inulin to detect their influence on its sensory, chemical, microbial quality, and shelf life. Moreover, studying the antimicrobial effect of *L. plantarum* and inulin on the survival of *E. aerogenes*, as well as investigating the effect of inulin on the viability of *L. plantarum* in cheese during the storage period.

Materials and Methods

Materials

Fresh buffalo's skim milk was obtained from the Faculty of Agriculture, Cairo University. Chemical parameters of the used milk were determined using a milk analyzer (LCD display- 4 lines × 16 characters, 100-240V-1.6A max., Bulgaria); Commercial starter culture was purchased from Chr. Hansen (White Daily 41) Freeze Dried- Direct Vat Set (FD-DVS) contained *Lactobacillus delbrueckii* ssp. *Bulgaricus*, *Lactococcus lactis* ssp. *Lactis*, *Lactococcus lactis* ssp. *Cremoris*, and *Streptococcus thermophilus*. Strains of *L. plantarum* DSM 20174 and *E. aerogenes* DSM 30053 were obtained from Cairo-MIRCEN, Faculty of Agriculture, Ain-Shams University. Food grade fine salt from El-Nasr Salines Company, Egypt. Calcium chloride from Sigma Chemical Company, Str. Louis, USA. Microbial rennet powder (Reniplus 2000 IMCU) from Caglio Star, Proquiga, Spain) and Inulin (Orafti®, Belgium) were used.

Strains preparation

Lactobacillus plantarum was cultivated in MRS broth for 24 hours/37°C then enriched in sterile skimmed milk for 24 hours/37°C to reach approximately 9 $\log_{10}$cfu/g that was determined by plating serial dilutions on De Man, Rogosa, and Sharpe agar (MRS agar HIMEDIA). *Enterobacter aerogenes* was activated in tryptic soy broth (TSB) and incubated at 37°C/24 hours to reach a final concentration of approximately 8 $\log_{10}$cfu/g which was counted by plating serial dilutions on MacConkey agar (Oxoid) supplemented with 50 µg /ml nalidixic acid.

Karish cheese preparation

Karish cheese was manufactured as follows: the fresh milk was laboratory pasteurized at 74°C/15 seconds then suddenly cooled. Starter culture, rennet, and calcium chloride were added at 37°C according to the manufacturer instructions. The milk was left till coagulation (40–45 minutes), then the curd was scooped into mats similar to those used in the traditional technology to be like the shape of traditional cheese. Dry salt (2.5 g/100 g cheese) was sprinkled on the surface of the curd and left to drain. The resultant cheese was cut and packed in airtight plastic containers containing salted whey (2.5% salt) then stored at 4°C (Ahmed *et al*., 2005).

Experimental design

Part 1: study the effect of using *L. plantarum* with and without inulin on Karish cheese quality

Three separate Karish cheese treatments were made: control (C) treatment that prepared exactly like the previously mentioned steps in cheese preparation, (P) treatment prepared as control with inoculation of *L. plantarum* at a ratio of (2%, *v/v*), and (PI) treatment was done as control with the addition of both *L. plantarum* and 4% inulin. Samples were examined at zero, 3, 7, 14, 21, 28, and 35 days or till the appearance of spoilage sign(s) to determine the sensory, chemical, microbial quality, and shelf life of prepared cheeses. The experiments were done in triplicate.

Sensory evaluation

Sensory evaluation was carried out according to the American Dairy Science Association scorecard scheme described by Bodyfelt and Potter (2009) in which sensory

panelists were asked to provide an evaluation score as following: flavor (1–10 points, where 1 = extremely undesirable and 10 = excellent), body and texture (1–5 points, where 1 = extremely undesirable and 5 = excellent), and color and appearance (1–5 points, where 1 = extremely undesirable and 5 = excellent). The overall grade (20) was calculated from (100), (20 = 100%). Ten experienced panelists (from both sexes in the age range of 25–55 years) were chosen from the Food Hygiene and Control department staff members at the Faculty of Veterinary Medicine, Cairo University, Egypt. The prepared Karish cheese samples were cut, placed on white plates, and presented to the panelists randomly. Water was provided for mouth washing between samples.

Chemical analysis

Titratable acidity and moisture % were done according to guidelines of the Association of Official Analytical Chemists (AOAC, 2000).

Microbiological analysis

Presumptive coliform, staphylococci, yeast, and mold counts according to the guidelines of the American Public Health Association (APHA, 2004). Furthermore, the survival of *L. plantarum* was investigated.

Part 2: study the antimicrobial effect of *L. plantarum* with and without inulin on the survival of *E. aerogenes* in Karish cheese

Three separate Karish cheese treatments were made: control treatment that inoculated with *E. aerogenes* **(**CE). The second was (PE) treatment which contained (*E. aerogenes* + *L. plantarum*). The third was (PIE) treatment that contained (*E. aerogenes* + *L. plantarum* + 4% inulin). Samples were examined at 0, 3, 7, 14, and 21 days or till the appearance of spoilage sign (s) to determine the survival of *E. aerogenes* and *L. plantarum* as well as chemical parameters were measured. The experiments were carried out in triplicate.

Statistical analysis

All measurements were carried in triplicates; the results were expressed as mean ± standard error (SE). Data were also statistically analyzed by Analysis of variance using SPSS 23 for windows. Multiple comparisons of means were made using the least significant difference at the significance level ($p < 0.05$).

Ethical approval

In this study, we did not use any experimental live animals.

Results

Chemical analysis of buffalo milk used in the current study

The compositional analysis of used buffalo milk in this study was as following: fat (0.15%), S.N.F (9.39%), protein (3.48%), lactose (5%), titratable acidity (0.16%), and (pH 6.6.)

Part 1: effect of using L. plantarum with and without inulin on Karish cheese quality

Sensory evaluation

Data presented in Table 1 shows that all sensorial attributes were significantly higher in (P) and (PI) treatments than in control. The highest mean flavor

Table 1. Sensory evaluation of Karish cheese treatments (Mean ± SE).

Storage days		0 time	3 days	7 days	14 days	21 days	28 days	35 days
Flavor (1–10)	C	7.5 ± 0.2^{Aa}	7.7 ± 0.1^{Aa}	7.3 ± 0.1^{Aa}	6 ± 0.6^{Ab}	S*	–	–
	P	9.7 ± 0.1^{Bab}	10 ± 0^{Ba}	9.5 ± 0.2^{Bab}	9.2 ± 0.3^{Bab}	9 ± 0.3^{Ab}	8 ± 0.6^{c}	S
	PI	9.7 ± 0.1^{Ba}	9.8 ± 0.1^{Ba}	9.3 ± 0.1^{Bab}	9.1 ± 0.2^{Bb}	8.9 ± 0^{Ab}	S	–
Body and texture (1–5)	C	3.8 ± 0.1^{Aa}	3.5 ± 0.2^{Aab}	3.2 ± 0.1^{Aab}	2.8 ± 0.4^{Ab}	S	–	–
	P	4.5 ± 0.2^{ABab}	4.8 ± 0.1^{Ba}	4.6 ± 0.1^{Ba}	4.5 ± 0.2^{Bab}	3.8 ± 0.1^{Abc}	3.5 ± 0.3^{c}	S
	PI	4.8 ± 0.1^{Ba}	5 ± 0^{Ba}	5 ± 0^{Ba}	4.7 ± 0.1^{Ba}	4 ± 0.3^{Bb}	S	–
Color and appearance (1–5)	C	4.2 ± 0.1^{Aa}	4 ± 0^{Aa}	3.7 ± 0.1^{Aa}	2.8 ± 0.4^{Ab}	S	–	–
	P	4.8 ± 0.1^{Ba}	4.8 ± 0.1^{Ba}	4.6 ± 0.1^{Bab}	4.5 ± 0.2^{Bab}	4.2 ± 0.1^{Abc}	3.7 ± 0.2^{c}	S
	PI	4.8 ± 0.1^{Bab}	5 ± 0^{Ba}	4.7 ± 0.1^{Bab}	4.3 ± 0.1^{Bbc}	4 ± 0.3^{Ac}	S	–
Overall grade (100)	C	77.5 ± 1.5^{Aa}	75.8 ± 1.7^{Aa}	70.8 ± 0.8^{Aa}	58.3 ± 2.4^{Ab}	S	–	–
	P	95 ± 2.5^{Bab}	98.3 ± 1.7^{Ba}	93.8 ± 1.2^{Bab}	91 ± 3^{Bbc}	85 ± 1.5^{Bc}	75.8 ± 2.2^{d}	S
	PI	96.7 ± 1.7^{Bab}	98.7 ± 0.7^{Ba}	94.8 ± 1.6^{Bab}	90.5 ± 2.5^{Bbc}	84.7 ± 3.2^{Bc}	S	–

C = Control Karish cheese; P = Karish cheese with *L. plantarum*; PI = Karish cheese with *L. plantarum* and inulin; S = Spoiled.

$^{A–B}$Values with different superscripts within the same column are significantly ($p < 0.05$) different.

$^{a–d}$Values with different superscripts within the same raw are significantly ($p < 0.05$) different.

was recorded on the third day in (P) treatment (10) followed by (PI) treatment (9.8) as compared to (C) treatment (7.7). Regarding body and texture, color, and appearance scores, (PI) treatment was the uppermost (5), followed by (P) treatment (4.8) on the third day of examination. The highest overall score for (PI) and (P) treatments was recorded on the third day; it was not significantly different, 98.7 and 98.3, respectively, but significantly different from control 75.8.

Microbiological analysis

Presumptive coliform, staphylococci, yeast, and mold counts were not detected (<10 cfu/g) over the entire storage time except yeast appears in (C) treatment at day 21 with mean 1.60 $\log_{10}$cfu/g.

Part 2: The antimicrobial effect of L. plantarum with and without inulin on the survival of E. aerogenes in Karish cheese treatments

Data presented in Table 2 reveals that there was a significant reduction in *E. aerogenes* count started from third day in (PIE) (6.23 $\log_{10}$cfu/g) and (PE) (6.25 $\log_{10}$cfu/g) treatments compared to (CE) treatment (8.48 $\log_{10}$cfu/g). The log reduction rate reached to 3.76 $\log_{10}$cfu/g on the seventh day of storage in (PIE) compared to (CE) treatment. *E. aerogenes* count is significantly decreased in PIE and PE treatments throughout the storage period till it reached (3.73 $\log_{10}$cfu/g) in (PIE) treatment and (3.74 $\log_{10}$cfu/g) in (PE) treatment at 21 days.

Effect of using inulin on the survival of L. plantarum during storage period in different Karish cheese treatments

As seen in Table 3, the addition of inulin significantly increase *L. plantarum* count in (PI) treatment (9.69 $\log_{10}$cfu/g) compared to (P) treatment without inulin (9 $\log_{10}$cfu/g) started on the third day and till the end of the storage period.

Chemical analysis of different Karish cheese treatments

There was a significant increase in acidity % in all Karish cheese treatments (Table 4) within the storage period, the lowest acidity % obtained at zero time in (C) treatment (0.30), while the highest was obtained at 14 days in (PIE) treatment (1.03). The highest moisture content was obtained in treatments with 4% inulin (PI) (72.5) and (PIE) (72.3) at zero time till the end of the storage period compared to other treatments.

Discussion

Part 1: effect of using L. plantarum with and without inulin on Karish cheese quality

Sensory evaluation

Data presented in Table 1 illustrats the impact of using *L. plantarum* alone or with 4% inulin on the organoleptic properties of Karish cheese. Flavor scores of (P) and (PI) treatments were significantly higher than (C) treatment throughout the storage period. Graders described the flavor of (P) treatment as excellent. The

Table 2. Survival of *Enterobacter aerogens* in different Karish cheese treatments ($\log_{10}$ Mean ± SE).

Storage days	0 time	3 days	7 days	14 days	21 days
CE	7.44 ± 0.02^{Aa}	8.48 ± 0.01^{Ab}	8.91 ± 0.02^{Ac}	S	-
PE	7.43 ± 0.05^{Aa}	6.25 ± 0.03^{Bb}	5.18 ± 0.01^{Bc}	3.74 ± 0.02^{Ad}	S
PIE	7.43 ± 0.05^{Aa}	6.23 ± 0.01^{Bb}	5.15 ± 0.01^{Bc}	3.73 ± 0.01^{Ad}	S

CE = Control Karish cheese with *Enterobacter aerogens*; PE = Karish cheese with *L. plantarum* and *Enterobacter aerogens*; PIE = Karish cheese with *L. plantarum,* inulin and *Enterobacter aerogens*; S = Spoiled.

$^{A–B}$Values with different superscripts within the same column are significantly ($p < 0.05$) different.

$^{a–d}$Values with different superscripts within the same raw are significantly ($p < 0.05$) different.

Table 3. Survival of *L. plantarum* in different Karish cheese treatments ($\log_{10}$ Mean ± SE).

Storage days	0 time	3 days	7 days	14 days	21 days	28 days	35 days
P	7.90 ± 0.03^{Aa}	9.00 ± 0.06^{Ab}	8.50 ± 0.01^{Ac}	8.30 ± 0.01^{Ad}	8.27 ± 0.01^{Ad}	7.93 ± 0.01^{a}	S
PI	7.93 ± 0.01^{Aa}	9.69 ± 0.02^{Bb}	8.62 ± 0.01^{Bc}	8.47 ± 0.01^{Bd}	8.29 ± 0.01^{Ae}	S	–
PE	7.90 ± 0.03^{Aa}	8.92 ± 0.01^{Ab}	8.30 ± 0.01^{Cc}	8.28 ± 0.01^{Ac}	S	–	–
PIE	7.94 ± 0.01^{Aa}	8.97 ± 0.01^{Ab}	8.47 ± 0.01^{Ac}	8.15 ± 0.03^{Cd}	S	–	–

$^{A–C}$Values with different superscripts within the same column are significantly ($p < 0.05$) different.$^{a–e}$Values with different superscripts within the same raw are significantly ($p < 0.05$) different.

P = Karish cheese with *L. plantarum*; PI =: Karish cheese with *L. plantarum* and inulin; PE = Karish cheese with *L. plantarum* and *Enterobacter aerogens*; PIE = Karish cheese with *L. plantarum,* inulin and *Enterobacter aerogens*; S = Spoiled.

Table 4. Chemical examination of Karish cheese treatments (Mean ± SE).

Storage days		0 time	3 days	7 days	14 days	21 days	28 days	35 days
Acidity%	C	0.30 ± 0.02^{Aa}	0.45 ± 0.03^{Ab}	0.60 ± 0.06^{Ac}	0.77 ± 0.03^{Ad}	S*	–	–
	P	0.40 ± 0.06^{ACa}	0.60 ± 0.06^{ABb}	0.70 ± 0.06^{ACbc}	0.80 ± 0.06^{Acd}	0.90 ± 0.06^{Ad}	0.92 ± 0.07^{d}	S
	PI	0.43 ± 0.03^{ACa}	0.47 ± 0.03^{Aa}	0.57 ± 0.03^{Aab}	0.70 ± 0.06^{Abc}	0.80 ± 0.06^{Ac}	S	–
	CE	0.60 ± 0.06^{BDa}	0.70 ± 0.06^{Ba}	0.90 ± 0.06^{Bb}	S	–	–	–
	PE	0.47 ± 0.03^{CDa}	0.60 ± 0.06^{ABab}	0.67 ± 0.03^{ACbc}	0.80 ± 0.06^{Ac}	S	–	–
	PIE	0.60 ± 0.06^{BDa}	0.70 ± 0.06^{Ba}	0.80 ± 0.06^{CBa}	1.03 ± 0.0^{Bb}	S	–	–
Moisture%	C	69.40 ± 0.59^{Aa}	69.34 ± 0.67^{Aa}	69.33 ± 0.67^{Aa}	69.13 ± 0.55^{Aa}	S	–	–
	P	70.27 ± 0.73^{Aa}	70.17 ± 0.61^{Aa}	70 ± 0.58^{Aa}	69.87 ± 0.61^{Aa}	69.57 ± 0.50^{Aa}	69.50 ± 0.35^{a}	S
	PI	72.53 ± 0.41^{Ba}	72.43 ± 0.64^{Ba}	72.33 ± 0.79^{Ba}	72.23 ± 0.44^{Ba}	72 ± 0.58^{Ba}	S	–
	CE	69.37 ± 0.59^{Aa}	69.27 ± 0.44^{Aa}	69 ± 0.25^{Aa}	S	–	–	–
	PE	69.97 ± 0.59^{Aa}	69.70 ± 0.53^{Aa}	69.63 ± 0.62^{Aa}	69.43 ± 0.47^{Aa}	S	–	–
	PIE	72.33 ± 0.45^{Ba}	72.27 ± 0.36^{Ba}	72.10 ± 0.59^{Ba}	71.80 ± 0.64^{Ba}	S	–	–

$^{A–D}$Values with different superscripts within the same column are significantly ($p < 0.05$) different.

$^{a–d}$Values with different superscripts within the same raw are significantly ($p < 0.05$) different.

C = Control Karish cheese; P = Karish cheese with *L. plantarum*; PI = Karish cheese with *L. plantarum* and inulin; CE = Control Karish cheese with *Enterobacter aerogens*; PE = Karish cheese with *L. plantarum* and *Enterobacter aerogens*; PIE = Karish cheese with *L. plantarum,* inulin and *Enterobacter aerogens*; S = spoiled.

addition of lactobacilli as *L. plantarum* into cheese can significantly influence cheese proteolysis that is carried out through hydrolysis of caseins into peptides by milk enzymes, rennet, starter culture, and probiotic enzymes. Furthermore, the formed peptides are extra hydrolyzed by probiotic enzymes into smaller peptides and free amino acids that play a principal role in developing cheese flavor and texture (Karimi *et al*., 2012). Results agreed with those obtained by El-Shafei *et al*. (2008) and Allam *et al*. (2017a). Unlike other fat replacers, inulin can form a stable creamy-like gel without negatively affecting cheese flavor (Karimi *et al*., 2015). The current results were in accordance with Arcia *et al*. (2011) and Modzelewska-Kapituła *et al*. (2007), who reported that inulin improves cheese's sensorial properties, especially flavor. However, Franck (2002), Giri *et al*. (2017) found that the typical flavor of cheese decrease with increasing the amount of inulin as it gives a sweet taste affects cheese flavor.

Concerning body and texture, color, and appearance scores, (PI) treatment was the uppermost, followed by (P) treatment as demonstrated in Table 1. Inulin effectively achieves textural adjustment through acting as a bulking and texturizing agent (Tufeanu and Tița, 2016; Junyusen *et al*., 2017). The softening effect of inulin could be attributed to its higher affinity to absorb moisture (Koca and Metin, 2004; Karimi *et al*., 2015). Inulin results agreed with those obtained by Alnemr *et al*. (2013) in Karish cheese and with Salvatore *et al*. (2014) and Junyusen *et al*. (2017) in reduced-fat fresh cheese. Furthermore, Da Cruz *et al*. (2009) and Tufeanu and Tița (2016) reported that inulin and *Lactobacillus* improved the organoleptic profile of reduced-fat-cheeses. Additionally, Abou Ayana and Ibrahim (2015) reported that LAB improved the texture of Karish cheese.

The overall grade results of (P) and (PI) treatment reflected the success of *L. platarum* and inulin 4% in enhancing the organoleptic characteristics of Karish cheese, nearly similar to the conventional cheese made by farmers but with higher hygienic quality and capable of delivering specific health benefits.

Microbiological analysis and shelf life

Presumptive coliform, staphylococci, yeast, and mold counts were not detected (<10 cfu/g) over the entire storage time except yeast appears in (C) treatment at day 21 with mean 1.60 $\log_{10}$cfu/g; however, these results were within the acceptable limit of Egyptian standards (2005) (Not exceed 2.6 $\log_{10}$cfu/g). Heat treatment of cheese milk and using *L. plantarum* and inulin improved the hygienic quality of Karish cheese. Those results were in harmony with those obtained by Lavermicocca *et al*. (2000), El-Shafei *et al*. (2008), Allam *et al*. (2017b), and Ali *et al*. (2020).

In regards to shelf life, Karish cheeses prepared using *L. plantarum* (P) recorded the highest shelf life (28)

days followed by PI treatment (21 days), while (C) treatment was the lowest (14) days. The obtained results reflected the ability of *L. plantarum* to prolong the shelf life of Karish cheese, which usually ranges from 1 to 2 weeks (Hammam *et al*., 2020). This prolonged shelf life is owed to the ability of *L. plantarum* to produce bacteriocins, hydrogen peroxide, and organic acids, which hinder spoilage microbial growth (Dinev *et al*., 2018).

Part 2: The antimicrobial effect of L. plantarum with and without inulin on the survival of E. aerogenes in Karish cheese treatments

There was a significant reduction in *E. aerogenes* count in (PIE) and (PE) started from the third day then decreased significantly ($p < 0.05$) during the storage period (Table 2). Using Lactobacilli as an alternative to chemical preservatives has acquired much attention nowadays (Gupta and Srivastava, 2014; Shekh *et al*., 2016; Dinev *et al*., 2018). Several researchers reported the unique antimicrobial characters of *L. plantarum* against broad-spectrum Gram-positive and Gram-negative bacteria. It can produce bacteriocins, hydrogen peroxide, and organic acids (Dinev *et al*., 2018). The current results in accordance with those of Tambekar and Bhutada (2010), Shekh *et al*. (2016), Al-Gamal *et al*. (2019), Ali *et al*. (2020), and Vataščinová *et al*. (2020), who confirmed that *L. plantarum* had displayed antibacterial activity against *E. aerogenes, E. coli*, *Salmonella enteritidis*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Listeria ivanovii*, *S. aureus, Bacillus cereus,* and *Candida albicans*.

Effect of using inulin on the Survival of L. plantarum during the storage period

The addition of inulin (4%) significantly increases *L. plantarum* count in (PI) treatment compared to (P) treatment without inulin throughout the chilled storage period. The growth rate of *L. plantarum* increased during the storage of Karish cheese. It decreased gradually until the end of the storage period; this reduction may be related to intensive lactic acid production and refrigerated storage temperature (Karimi *et al*., 2015). However, it survived well in all inoculated treatments at the recommended level of 7.0 $\log_{10}$cfu/g, which required probiotics to deliver its benefits to humans (Hammam and Ahmed, 2019). Those results were in parallel with those found by Modzelewska-Kapituła *et al*. (2007).

Chemical evaluation of Karish cheese treatments

The highlighted results in Table 4 including that there was a significant development in acidity % in all treatments during storage days, a slight significant ($p < 0.05$) increase in treatments containing *L. plantarum*, and *E. aerogenes* within storage time due to acid production from both bacteria. Rapid gas and acid production from lactose fermentation is considered a diagnostic feature of *E. aerogenes* (Baylis, 2006). Regarding moisture %, it was significantly affected when using inulin in (PI) and (PIE) treatments owing to water absorbability of inulin and consequent increase in the moisture content. The finding is consistent with the experiments of Alnemr *et al*. (2013) in Karish cheese and Juan *et al*. (2013) in reduced-fat fresh cheese.

Conclusion

Outcomes of this study clarified that the addition of *L. plantarum* and inulin into Karish cheese synergistically enhanced its sensory characteristics, hygienic quality, and shelf life. Moreover, the addition of inulin positively affects the viability of *L. plantarum*. This study recommended using *L. plantarum* and inulin in pasteurized Kaish cheese to produce a safe product nearly similar to the traditional one with new functional synbiotic properties.

Conflict of interest

The Authors declare that there is no conflict of interest.

Author's contribution

Ashraf A. Moawad planned, supervised the study, and revised the final version of manuscript before submission. Eman F. Abdel-Latif shared in the study design, assisted in experiment performing, and reviewed the final manuscript. Hamdy M.B.A. Zaki and Ola W. Hegab collected the required materials, performed different experimental treatments, collect the data, interpreted and statically analyzed the obtained results, finally they drafted the manuscript.

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