

Journal of Food and Dairy Sciences

Journal homepage: www.jfds.mans.edu.eg

Available online at: www.jfds.journals.ekb.eg



Using *in vitro* Digestion Method in Assessing the Viability of *Lactobacillus* spp. in White Soft Cheese-Like Products

Aliaa A. Darwish*

Dairy Research Dept., Food Technology Research Institute, Agricultural Research Center, Giza, 12613, Egypt

ABSTRACT

Three cheese samples containing *L. casei* formulated with milk fat (C1), shortening oil (SH1), and cocoa butter substitute oil (SU1) and other three samples containing *L. acidophilus* formulated with milk fat (C2), shortening oil (SH2), and cocoa butter substitute oil (SU2) were prepared. Cheese samples were evaluated for their chemical composition, pH, and viable counts of *L. casei* and *L. acidophilus* during storage for 30 days before and after *in vitro* digestion. pH values gradually decreased over time (milk fat-based cheese samples had the highest pH values). Results revealed that the viability of *Lactobacillus* spp. was strain-dependent (*L. casei* was of higher viability than *L. acidophilus*). In the undigested samples, the viability of *L. casei* and *L. acidophilus* significantly decreased over time, keeping higher viable counts ($>10^6$ CFU/g). *In vitro* digestion strongly declined *Lactobacillus* viability and *L. acidophilus* was the most inhibited strain. Results demonstrated a great influence of fat type on *Lactobacillus* viability where the highest viable numbers were observed in samples containing milk fat (C1, and C2). Moreover, the inhibition rate (%) was strongly influenced by *in vitro* digestion, fat type, and *Lactobacillus* strain used. Accordingly, the higher inhibition rate was recorded for *L. acidophilus* in digested cheese containing shortening and substitute oils (41.47, and 34.04%, respectively). Thus, the results of the current study indicated that milk fat is the most suitable fat type in protecting probiotic viability in cheese.

Keywords: Probiotic viability, *L. casei* and *L. acidophilus*, White soft cheese-like products, Palm oil, *in vitro* digestion

INTRODUCTION

Cheese is one of the most important elements of the human diet in developed and developing countries alike. Currently, manufacturers tend to minimize cheese production cost and thus increasing competitiveness and gaining more profit by replacing milk fat with some vegetable oils (Aljewicz *et al.*, 2014). These oils are inexpensive, as compared to milk fat, and have enhancing effects on the quality and stability of the resulting cheese-like products. Thus, cheese containing vegetable oils are widely produced around the world and in Egypt as well.

Recently, cheese has increased in popularity due to its suitability in delivering probiotics to human (Karimi *et al.*, 2011). Thus, the production of cheese containing probiotic bacteria greatly meets the needs of the modern consumer and the demands of the changing market (Cichosz *et al.*, 2014). Interestingly, the cheese matrix is considered one of the most important factors that affect probiotic viability in cheese during storage and ripening. Regarding this, several strains of probiotic bacteria have been investigated for assessing their viability in fresh cheese (Masuda *et al.*, 2005), and fresh cheese with inulin (Buriti *et al.*, 2007), and semi-hard cheese (Aljewicz *et al.*, 2014). The later author observed lower viability of *Lactobacilli* spp. in Gouda cheese prepared with palm oil as a milk fat substitute. According to the results of the study conducted by Rodrigues *et al.* (2012), probiotic viability is deeply influenced by the fat type and liberated fatty acids during cheese storage and ripening. Moreover, probiotic bacteria lose a portion of their viability while they passing in the gastrointestinal tract because they are exposed to digestive enzymes and pH conditions. In this sense, *in vitro*

digestion model has been widely used as a suitable alternative for *in vivo* studies. Thus, the most common method of *in vitro* digestion used as an alternative to *in vivo* studies is that which uses pure enzymes including pepsin, and pancreatin (Council of Europe, 2004; United States Pharmacopeial Convention, 2006).

Nowadays, several studies have been currently carried out to evaluate the functionality of cheese by exposing it to *in vitro* digestion to estimate its generated bioactive peptides. Nevertheless, few studies have been aimed to estimate the viability of probiotics existent in a cheese after *in vitro* digestion.

Assessing the viability of probiotic culture after *in vitro* digestion is of great importance to providing health benefits associated with probiotic bacteria. However, there are no studies conducted to determine probiotic survivability in Egyptian white soft cheese-like products (that containing high amounts of vegetable oils) after *in vitro* digestion. Consequently, the objective of the present study was to assess the viability of two *Lactobacillus* strains (*L. casei* and *L. acidophilus*) in milk fat-, shortening oil-, and cocoa butter substitute oil-based cheese samples during cold storage and after *in vitro* digestion.

MATERIALS AND METHODS

Raw materials, and chemicals

Skimmed fresh cow milk (0.1 % Fat and 3.4% protein) and raw heavy cream (68% fat) were obtained from Dairy Technology Unit, Faculty of Agriculture, Cairo University (Giza, Egypt). Skimmed milk powder (0.1% fat, and 36% protein) was obtained from Valio Ltd. (Helsinki, Finland). Glucono delta lactone (GDL) was obtained from Shandong Kaison Biochemical Co., Ltd., Wulian Country Shandong (China).

* Corresponding author.

E-mail address: aliaa-2006-55@hotmail.com

DOI: 10.21608/jfds.2021.69630.1019

Microbial rennet (Reniplus 2000 IMCU) was obtained from CAGLIO STAR, Proquiga Biotech., S.A., Murcia (Spain). Dry fine grade table salt (NaCl) was obtained from El-Nasr for salt production Co., Alexandria (Egypt) while calcium chloride was obtained from Alpha Chemik (India). Textra Tallaga 300 stabilizer was obtained from AWA Food Solutions Company, Alexandria (Egypt). Shortening oil was produced by Arma Food Industries Company and kindly provided by Green Fields Company for dairy products, Kafrelsheik (Egypt) while cocoa butter substitute oil (hydrogenated palm kernel oil, NCOTE 347) was produced by Premium vegetable oils Sdn Bhd, Kuala Lumpur (Malaysia) and kindly provided by Healthy Milk Company for dairy products, Elsharkia (Egypt). Enzymes and bile salts were purchased from Sigma Chemical Co. (St. Louis MO, USA): Pepsin (Porcine: cat. no. P-7000), Pancreatin (Porcine: cat. no. B-8756), and bile salt (Porcine: cat. no. P-1750). All other solvents, chemicals, and culture media (MRS) were of analytical grade.

Fatty acids composition

According to ISO 12966:2 (2017), fatty acids profile in milk fat, shortening oil, and cocoa butter substitute oil used in the preparation of the various samples of cheese has been determined where the fatty acids were converted into their methyl esters (FAMEs) using a cold saponification method.

Propagation of *Lactobacillus* strains

Two probiotic strains (*L. casei* and *L. acidophilus*) were kindly provided by Food Science and Human Nutrition Dept. (NUTBRO group), Faculty of Veterinary Sciences, University of Murcia, Spain. These two strains were activated twice in MRS broth for 48 h at 37°C under anaerobic conditions. The propagated cell suspensions were cultured in sterilized cow's skimmed milk and anaerobically incubated at 40 ± 1 °C until curdling of the milk and then stored at 4 °C (Rashid *et al.*, 2007). On the next day, they were used as adjunct culture in white soft cheese production.

White soft cheese processing

Cheese and cheese-like product samples were formulated using fresh skimmed milk, skimmed milk powder, and source of fat (raw heavy cream, shortening oil, or cocoa butter substitute oil). All samples were prepared to contain 6% protein and 25% fat. White soft cheese (two formulations) and white soft cheese-like products (four formulations) were produced at laboratory scale at Dairy Research Dept., Food Technology Research Institute, Giza, Egypt. Fig. 1 presents the flow diagram of the processing steps of white soft cheese and white soft cheese-like products using the non-traditional method without whey drainage.

The six experimental treatments studied in the current research were as follows:

Milk fat-based cheese with *L. casei* named C1, shortening-based cheese with *L. casei* named SH1, and cocoa butter substitute oil-based cheese with *L. casei* named SU1.

Milk fat-based cheese with *L. acidophilus* named C2, shortening-based cheese with *L. acidophilus* named SH2, and cocoa butter substitute oil-based cheese with *L. acidophilus* named SU2.

The resultant cheese samples were kept for 30 days at 5°C in plastic containers (capacity 150g). The experiments were carried out in triplicate. Each sample has been analyzed at 1, 15, and 30 days of storage. Contents of total solids, fat, fat/dry matter, protein, salt, salt in moisture, and ash were detected in each sample on the first day of cold storage while

pH values were recorded at 1, 15, and 30 days. The viable numbers of *Lactobacillus* strains were counted at 1, 15, and 30 days before and after *in vitro* digestion process.

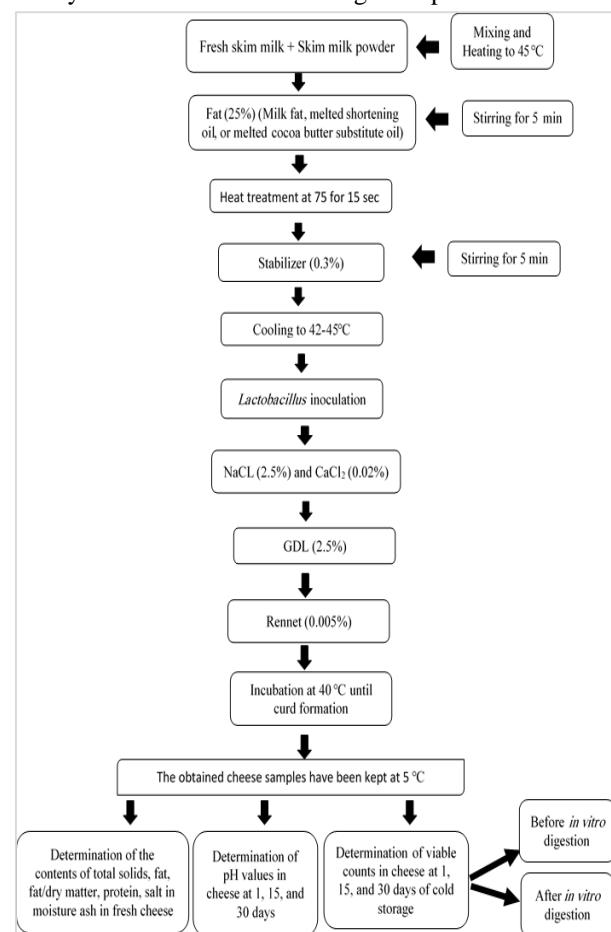


Fig. 1. The flow diagram of the processing steps of white soft cheese and white soft cheese-like products using the non-traditional method without whey drainage.

Chemical composition of cheese

The contents of total solids, fat, fat/dry matter, protein, and ash were determined in cheese samples as described by AOAC (2012). The pH of the samples was monitored by pH meter (Crison Instruments, Spain) according to Torre *et al.*, (2003). The salt content of each cheese sample has been estimated according to AOAC (2003). Salt in moisture (%) was calculated using the following equation:

$$\text{salt/moisture (\%)} = 100 \times (\text{salt content \%} / \text{cheese moisture \%}).$$

In vitro digestion

White soft cheese samples were digested using the simplest protocol of *in vitro* digestion at 37°C according to Cattaneo *et al.* (2017) with some modifications. Briefly, the simulated *in vitro* digestion was divided into two phases: the gastric phase and the intestinal phase. For the gastric phase, 4.5 g of each cheese sample was well-mixed with 30 mL of deionized water. The gastric digestion has been conducted at pH 2 (adjust pH of this solution to 2 by using 1 M HCl). Then, an amount of freshly prepared pepsin solution, sufficient to yield 0.02 g pepsin/g of cheese sample, was added. The sample was incubated in a shaking water bath at 37 °C and 120 strokes/min for 2 h. Afterward, pepsin was inactivated by keeping the sample container in cold water at 5°C. For intestinal digestion, the pH of the gastric digestate was raised to pH 7.0 by dropwise addition of 1 M NaHCO₃. Then an

amount of freshly prepared pancreatin/bile solution, sufficient to provide 0.005 g of pancreatin and 0.03 g bile salts/g of cheese, was added, and the incubation was continued for an additional 2 h at 37 °C. The obtained digestate was used immediately in microbiological analysis.

Microbiological analysis

All cheese samples (before and after digestion) have been subjected to microbiological analysis at 1, 15, and 30 days of cold storage according to Najgebauer-Lejko (2014). Ten grams of the undigested cheese samples were suspended in 90 mL sodium citrate solution (2%, w/v) while the 10 g of digested samples (obtained after *in vitro* digestion) were suspended in 90 mL of sterilized saline solution (0.9%, w/v). Samples were homogenized using vortex and serially diluted using sterilized saline solution. The viable counts of *L. casei* and *L. acidophilus* in all cheese samples were counted in MRS-agar using the pour plate technique. The plates were anaerobically incubated at 37 °C for 72 h and the viable counts were expressed as a colony-forming unit (CFU) per gram of cheese. Also, the inhibition rate percent of each probiotic strain was calculated according to the following equation:

$$\text{Inhibition rate (\%)} = \frac{100 \times (\text{bacterial count on the first day} - \text{bacterial count on the final day})}{\text{bacterial count on the first day}}$$

Cheese samples were also checked for the presence of molds, and yeasts according to Ousman *et al.* (2008).

Statistical analysis

Statistical analysis was carried by analyzing the obtained data by a one-way analysis of variance (ANOVA) with SPSS v20 software (IBM Corp., Armonk, NY, USA), and the comparison between means was done by Tukey's test at $p < 0.05$. The data are expressed as means \pm SD.

RESULTS AND DISCUSSION

Fatty acids composition in milk fat, shortening, and substitute

The fatty acid profile in the milk fat, shortening oil, and cocoa butter substitute oil is shown in Table 1. Results indicated that milk fat had 71.56% saturated fatty acids, 26.49% monounsaturated fatty acids, and 2.12% polyunsaturated fatty acids. In quantitative terms, palmitic acid is the most abundant fatty acid whose content was 31.57% followed by stearic (C18:0) and myristic (C14:0) acids which present in concentrations of 12.08, and 11.72%, respectively. Regarding short-chain fatty acids, its content was 9.94% of the saturated fatty acids. Medium-chain fatty acids constitute 16.62% while long saturated fatty acids were found in a concentration of 44.82%. Oleic acid (C18:1) is considered the most abundant monounsaturated fatty acid (its content is 23.83%) in milk fat while linoleic acid (C18:2) and α -linolenic acid (C18:3n3) were the main polyunsaturated fatty acids (their contents were 1.68 and 0.44%). In line with the current results, Lindmark-Mansson (2003) pointed out a similar fatty acid profile in milk fat.

Unlike milk fat which contains butyric acid as a unique short-chain fatty acid, the short-chain fatty acids (butyric, caproic, caprylic, and capric acids) were absent in shortening oil while cocoa butter substitute oil contains lower amounts of caproic (0.31%), and higher amounts of caprylic, and capric acids (4.42, and 3.61%, respectively). Besides, shortening oil was characterized by the presence of a high concentration of saturated fatty acids (more than 50%), mainly palmitic acid (45.25%), and lower amounts of stearic

acid (4.30%) while cocoa butter substitute oil has been ascribed by the extremely existence of saturated fatty acids (~90%), mostly lauric acid (42.07%), stearic acid (18.32%), myristic acid (13.18%), and palmitic acid (9.10%). Also, data showed that oleic acid was the most existed unsaturated fatty acid in shortening oil (its content was 38.16%) while cocoa butter substitute oil contained small amounts (4.09%) of oleic acid. As expected, the polyunsaturated fatty acids (PUFAs) did not exist in cocoa butter substitute oil while shortening oil contains almost 9.91% of PUFAs, mainly linoleic acid (9.62%). Generally, similar results regarding the profile of fatty acids present in shortening oil and cocoa butter substitute oil were previously reported by several studies (Bakeet *et al.*, 2013; Abd El-Gawad *et al.*, 2015; Devi and Khatkar, 2018).

Table 1. Fatty acid composition of milk fat, shortening oil, and cocoa butter substitute oil used in the preparation of white soft cheese and white soft cheese-like products.

Fatty acids (%)	Milk fat	Shortening oil	Cocoa butter substitute oil
Butyric (C4:0)	4.25	-	-
Caproic (C6:0)	2.36	-	0.31
Caprylic (C8:0)	1.19	-	4.42
Capric (C10:0)	2.14	-	3.61
Lauric acid (C12:0)	2.6	0.24	42.07
Tridecanoic acid (C13:0)	0.85	0	0.05
Myristic acid (C14:0)	11.72	1.07	13.18
Pentadecanoic (C15:0)	1.45	-	0.01
Palmitic acid (C16:0)	31.57	45.25	9.1
Heptadecanoic (C17:0)	0.9	0.1	0.03
Stearic acid (C18: 0)	12.08	4.3	18.32
Arachidic acid (C20:0)	0.27	0.35	0.22
Behenic acid (C22:0)	0.18	0.06	0.04
Saturated fatty acids (SFA) Total	71.56	51.37	91.36
Myristoleic acid (C14:1)	0.49	-	-
Palmitoleic (C16:1)	1.76	0.21	-
Heptadecenoic acid (C17:1)	0.31	0.03	-
Oleic acid (C18:1)	23.83	38.16	4.09
Eicosenoic acid (C20:1)	0.1	0.13	0.02
Monounsaturated fatty acids (MUFA), cis, Total	26.49	38.53	4.11
Linoleic acid (C18: 2)	1.68	9.62	0.11
α -linolenic acid (C18: 3n3)	0.44	0.22	-
gamma-linolenic acid (C18: 3n6)	-	0.07	-
Polyunsaturated fatty acids (PUFA), cis, Total	2.12	9.91	0.11
Elaidic acid (C18: 1 T)	-	0	4.44
Linolelaidic (C18: 2 T)	0.7	0.2	-
Trans fatty acids, Total	0.7	0.2	4.44
Short chain fatty acids (SCFA)	9.94	0	8.34
Medium-chain fatty acids	16.62	1.31	55.31
Long saturated chain fatty acids (LCFA)	44.82	50	27.67

Moreover, results revealed that cocoa butter substitute oil contains a low level (4.44%) of the Trans fatty acid named elaidic acid (C18:1T) while this it was absent in shortening oil. The presence of this trans fatty acid in cocoa butter substitute oil could be due to the hydrogenation process that the oil was exposed to. The milk fat contains 0.7% of the Trans fatty acid named linolelaidic acid (C18:2T) which could be created by the action of the rumen microorganisms. Generally, the existence of Trans fatty acids in the used oils had several harmful effects on human health. The finding of the study carried out by Ratnayake *et al.* (1998) supported this observation.

Composition of white soft cheese

Data presented in Table 2 showed the chemical composition of white soft cheese and white soft cheese-like

products prepared by the non-traditional method using milk fat, shortening oil, or cocoa butter substitute oil. Results revealed that all treatments had almost similar contents of the estimated parameters (total solids, fat, fat/dry matter, protein, salt in moisture, and ash). Insignificant changes have been observed in total solids (ranged between 44.54 and 45.37%), fat (ranged between 24.86 and 24.96%), fat/dry matter (ranged between 54.91 and 55.96%), protein (ranged between 5.92 and 6.06%), and salt in moisture (ranged between 4.43 and 4.70%) while significant changes were observed in ash content values (ranged between 3.39 and 3.83%). The results of this study meet the requirements of the Egyptian standard

specification (1867/2005), which states that the proportion of fat in dry matter of the white soft cheese must be in the range of 40-60%. The observed close values reported for fat, fat/dry matter, protein, and salt in moisture could be attributed to the previous adjustment of cheese composition associated with its contents of protein, fat, and salt. The slight variation observed between treatments could result from the difference in the fat type used in cheese preparation and its ability to hold water. In line with these results, several studies reported almost similar chemical composition of white soft cheese (Ismail *et al.*, 2010; and Abd El-Halim *et al.*, 2007).

Table 2. Chemical composition (%) of fresh white soft cheese and white soft cheese-like product samples prepared using milk fat, shortening oil, and cocoa butter substitute oil.

Parameter (%)	Total solids	Fat	Fat/dry matter	Salt in moisture	Protein	Ash
C1	44.54 ± 0.31 ^a	24.92 ± 0.33 ^a	55.96 ± 1.04 ^a	4.43 ± 0.05 ^a	5.99 ± 0.13 ^a	3.51 ± 0.05 ^{cd}
SH1	45.27 ± 0.40 ^a	24.96 ± 0.33 ^a	55.14 ± 1.02 ^a	4.49 ± 0.03 ^a	5.95 ± 0.19 ^a	3.83 ± 0.09 ^a
SU1	44.75 ± 1.09 ^a	24.86 ± 1.03 ^a	55.54 ± 0.96 ^a	4.46 ± 0.15 ^a	5.94 ± 0.14 ^a	3.70 ± 0.09 ^b
C2	45.15 ± 0.07 ^a	24.93 ± 0.14 ^a	55.22 ± 0.36 ^a	4.70 ± 0.04 ^a	6.06 ± 0.19 ^a	3.39 ± 0.02 ^d
SH2	45.37 ± 0.46 ^a	24.91 ± 0.15 ^a	54.91 ± 0.76 ^a	4.59 ± 0.04 ^a	5.92 ± 0.11 ^a	3.57 ± 0.06 ^c
SU2	45.22 ± 0.43 ^a	24.94 ± 0.16 ^a	55.16 ± 0.64 ^a	4.43 ± 0.02 ^a	6.01 ± 0.18 ^a	3.57 ± 0.09 ^c

Values are means ± SD of three independent replicates. Means with different superscripts are significantly different ($p < 0.05$). C1 and C2: cheese samples prepared with milk fat and containing *L. casei*, and *L. acidophilus*. SH1, and SH2: cheese samples prepared with shortening oil and containing *L. casei*, and *L. acidophilus*. SU1, and SU2: cheese samples prepared with cocoa butter substitute oil and containing *L. casei*, and *L. acidophilus*. Fat/dry matter: % fat / % dry matter * 100. Salt in moisture = 100 * (salt % / moisture %).

pH values of soft cheese

Data exist in Fig. 2 displayed that the pH values of different cheese samples decreased over cold storage for 30 days. However, minor changes in pH values as impacted by storage time were observed. Regarding the influence of the fat type used and *Lactobacillus* strains added, slight variations were observed between pH values obtained for the cheese samples containing *L. casei* (C1, SH1, and SU1) and that containing *L. acidophilus* (C2, SH2, and SU2). Generally, pH values were slightly lower in cheese samples containing *L. acidophilus* (C2, SH2, and SU2). Moreover, cheese samples containing milk fat showed slightly higher values of pH. Similar pH values have been obtained by Foda *et al.* (1976) upon replacing milk fat with vegetable oils.

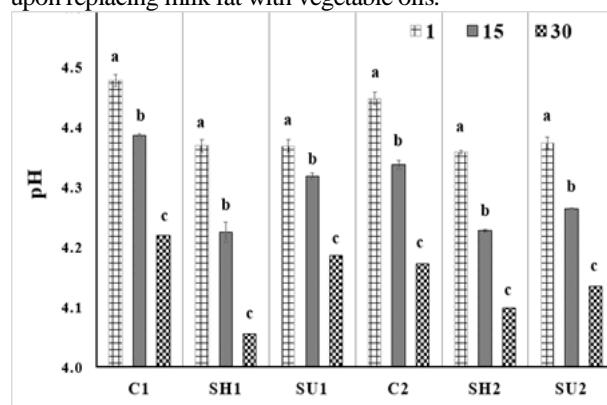


Fig. 2. pH values of white soft cheese and white soft cheese-like products samples prepared using milk fat, shortening oil, and cocoa butter substitute oil.

Values are means ± SD of three independent replicates. Column charts with different superscripts between times for the same sample are significantly different ($p < 0.05$). C1 and C2: cheese samples prepared with milk fat and containing *L. casei*, and *L. acidophilus*. SH1, and SH2: cheese samples prepared with shortening oil and containing *L. casei*, and *L. acidophilus*. SU1, and SU2: cheese samples prepared with cocoa butter substitute oil and containing *L. casei*, and *L. acidophilus*.

Viable counts and inhibition rate of *Lactobacillus* strains

Regarding the mold & yeast counts, they were not detected in all samples along the cold storage period due to the good hygienic conditions followed during sample preparation and storage.

Results exist in Tables 3, and 4 showed that counts of *L. casei* and *L. acidophilus* in white soft cheese and white soft cheese-like products significantly decreased during the cold storage period. However, these two strains maintained viable counts higher than the therapeutic level ($>10^6$ cfu/g) recommended and necessary to demonstrate its physiological effects. Similar data regarding probiotic viability in various cheese types were obtained by Ozer *et al.* (2008); Sharp *et al.* (2008); and Bergamini *et al.* (2009). The high viability of probiotics could result from the availability of nutrients in cheese especially at the initial phase of cold storage. The highest viable counts of *Lactobacillus* strains were observed in the presence of milk fat while substituting milk fat with cocoa substitute butter oil or shortening oil resulted in lower viable counts. Thus, the type of fat (fatty acid composition) might be a responsible factor in determining the survival rate of probiotic culture. Accordingly, the food matrix had a great influence on probiotic viability in white soft cheese. Similar trend has been obtained by Aljewicz *et al.* (2016) where substitution of milk fat with palm oil resulted in lower viable counts of *L. acidophilus NCFM* and *L. paracasei LPC-37* probiotic cultures in Gouda-type cheese-like products. Results presented in Tables 3 and 4 revealed that *L. casei* strain was more resistant than *L. acidophilus* suggesting that probiotic viability in cheese is strain-dependent. Generally, the inhibition observed for some probiotic cultures could result from the limited metabolic activity under low temperature (Aljewicz *et al.*, 2016) and inappropriate pH values along with the cold storage of cheese (Gomes and Malcata, 1998). For instance, the optimum pH for the growth of *L. acidophilus* is 5.5–6.0 (Gomes and Malcata 1998). This fact may explain the lower viable counts of *L. acidophilus* in

cheese as pH values ranged between 4.09–4.45 in cheese samples containing *L. acidophilus*.

Table 3. Viable counts (log CFU/g) of *L. casei* in white soft cheese and white soft cheese-like products samples prepared using milk fat, shortening oil, and cocoa butter substitute oil.

		Viable counts of <i>L. casei</i> (Log CFU/g)		
		1 day	15 days	30 days
Before digestion	C1	8.86 ± 0.01 ^{aA}	8.36 ± 0.07 ^{bA}	8.11 ± 0.09 ^A
	SH1	7.79 ± 0.10 ^{aC}	7.08 ± 0.18 ^{bC}	6.82 ± 0.07 ^{cC}
	SU1	8.04 ± 0.04 ^{aB}	7.33 ± 0.06 ^{bB}	7.23 ± 0.13 ^{bB}
After digestion	C1	6.92 ± 0.02 ^{aA}	6.37 ± 0.03 ^{bA}	6.23 ± 0.03 ^{aA}
	SH1	6.01 ± 0.09 ^{aC}	5.76 ± 0.15 ^{bC}	4.82 ± 0.11 ^{cC}
	SU1	6.36 ± 0.09 ^{aB}	6.13 ± 0.13 ^{bB}	5.11 ± 0.03 ^{cB}

Values are means ± SD of three independent replicates. Means with different superscripts (small letters) in the same rows while means with different superscripts (capital letters) in the same columns are significantly different ($p < 0.05$). C1: cheese samples prepared with milk fat and containing *L. casei*. SH1: cheese samples prepared with shortening oil and containing *L. casei*. SU1: cheese samples prepared with cocoa butter substitute oil and containing *L. casei*. CFU: colony-forming unit.

Table 4. Viable counts (log CFU/g) of *L. acidophilus* in white soft cheese prepared using milk fat, shortening oil, and cocoa butter substitute oil.

		Viable counts of <i>L. acidophilus</i> (Log CFU/g)		
		1 day	15 days	30 days
Before digestion	C2	8.80 ± 0.01 ^{aA}	8.18 ± 0.12 ^{bA}	7.41 ± 0.10 ^{aA}
	SH2	8.14 ± 0.06 ^{aC}	6.65 ± 0.16 ^{bC}	6.46 ± 0.15 ^{bC}
	SU2	8.28 ± 0.02 ^{aB}	6.98 ± 0.09 ^{bB}	6.89 ± 0.19 ^{bB}
After digestion	C2	6.76 ± 0.01 ^{aA}	5.34 ± 0.02 ^{bA}	5.23 ± 0.03 ^{aA}
	SH2	5.84 ± 0.06 ^{aC}	4.59 ± 0.11 ^{bC}	3.42 ± 0.10 ^{cC}
	SU2	5.97 ± 0.03 ^{aB}	4.84 ± 0.06 ^{bB}	3.94 ± 0.03 ^{cB}

Values are means ± SD of three independent replicates. Means with different superscripts (small letters) in the same rows while means with different superscripts (capital letters) in the same columns are significantly different ($p < 0.05$). C2: cheese samples prepared with milk fat and containing *L. acidophilus*. SH2: cheese samples prepared with shortening oil and containing *L. acidophilus*. SU2: cheese samples prepared with cocoa butter substitute oil and containing *L. acidophilus*. CFU: colony-forming unit.

Although the current study demonstrated a high viable population ($>10^6$ CFU/g) of *L. casei* and *L. acidophilus* in undigested samples (Tables 3 and 4), it does not ensure the same survival capacity for cells during their gastrointestinal passage. Thus, one of the most important factors that negatively influence probiotic viability is the transition along the gastrointestinal tract. This can be mainly explained by the decreased pH value in the stomach and the existence of bile salt in the small intestine (Mortazavian *et al.*, 2008).

Also, data in Tables 3 and 4 exhibited that *in vitro* digestion strongly decreased the viable counts of *L. casei* and *L. acidophilus*. This effect was more pronounced in *L. acidophilus* especially that present in cheese samples with shortening oil and cocoa butter substitute oil. Regarding *L. casei* viability, it was more resistant especially in cheese sample containing milk fat at various sampling points. Generally, the impact of *in vitro* digestion was more pronounced at 30 days of cold storage and less pronounced at the first days of cold storage.

Food matrix also likely plays a specific role in the changes that occurred in probiotic viability upon food digestion. Consequently, cheese containing milk fat had the highest viable counts followed by that contains cocoa butter substitute oil, and then that contains shortening oil. This observation could be explained by the changes in the fatty acid profile that occurred upon digestion. Similar to these results, Kashmir & Mankr

(2014) obtained lower viable counts of lactic acid bacteria in cheese-like products (that contained palm oil). Moreover, declined viability of lactic acid bacteria was observed in cheese-containing accumulated free fatty acids. This effect could be due to the formation of some metabolites including oxylipids and short-chain aldehydes participate in eliminating the bacterial cells through increasing the cell walls' permeability, inhibiting intracellular enzymes, and blocking ion channels that transport nutrients to bacterial cells.

Several factors are responsible for the antibacterial activity described for free fatty acids including chain length, acid structure, and double bonds number. In this sense, the results of the study conducted by Corcoran *et al.* (2007) indicated that α -linolenic acid (C18:3n3) exhibited bactericidal effects against lactic acid bacteria while elaidic acid (C18: 1T) promoted the growth of lactic acid bacteria. According to Greenway and Dyke (1979), the increase in polyunsaturated fatty acids highly reduced the viable counts of *Lactobacillus* sp. This could be associated with the unsaturation degree of the fatty acid. Hence, the greater the unsaturation degree of the fatty acid, the greater its toxicity (Kashmir & Mankr, 2014). However, the sensitivity of lactic acid bacteria to the accumulated free fatty acids might be varied within the same genus or even species of bacteria.

Accordingly, Corcoran *et al.* (2007) demonstrated that *Lactobacillus* spp. viability has been stimulated in the presence of oleic acid while their viability has been declined by linolenic acid. The former fatty acid inhibited the growth of lactic acid bacteria because of its high susceptibility to oxidation. The current results confirmed the negative effect of monounsaturated- and polyunsaturated fatty acids which highly present in shortening oil (its contents were 38.53 and 9.91%) on *Lactobacillus* viability. This negative effect could result from the toxic substances generated from fatty acids oxidation which have a damaging effect on bacterial membrane phospholipids. This toxicity is multiplied by increasing the unsaturation degree. For that reason, linolenic acid (C18:3) has greater inhibitory activity than linoleic acid (C18:2) on the growth of bacteria.

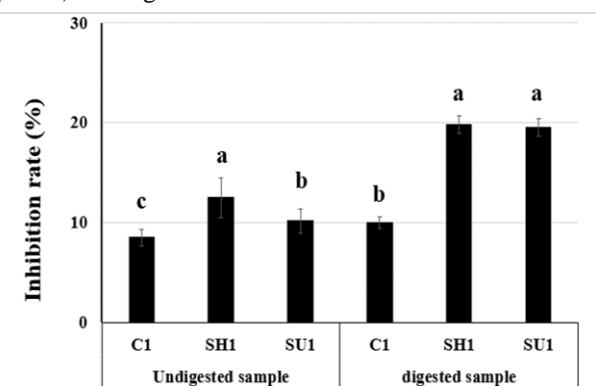


Fig. 3. Inhibition rate (%) of *L. casei* in white soft cheese and white soft cheese-like products prepared using milk fat, shortening oil, and cocoa butter substitute oil.

Values are means ± SD of three independent replicates. Column charts with different superscripts are significantly different ($p < 0.05$). C1: cheese samples prepared with milk fat and containing *L. casei*. SH1: cheese samples prepared with shortening oil and containing *L. casei*. SU1: cheese samples prepared with cocoa butter substitute oil and containing *L. casei*.

The results shown in Figs. 3 and 4 exhibited the inhibition rate (%) of *L. casei*, and *L. acidophilus*, respectively. Results confirmed that inhibition rate percent strongly influenced

by the digestion process, fat type, and *Lactobacillus* strain. So, the digested samples presented higher inhibition rate (lower viability) as compared to the undigested samples. Moreover, *L. casei* demonstrated a lower inhibition rate whether in digested or undigested samples, as compared to *L. acidophilus*. Regarding the impact of fat type, higher inhibition rates of 41.47, and 19.85% were recorded in shortening-based cheese (SH2, and SH1, respectively) followed by that containing cocoa butter substitute oil (SU2, and SU1) which their inhibition rates were 34.04, and 19.55%, respectively. While the lower inhibition rates (15.82, and 8.5%) were observed for cheese samples containing milk fat (C2, and C1, respectively). Thus, probiotic bacteria in cheese, especially white soft cheese prepared by the non-traditional methods using vegetable oils, must be protected to keep their viability and therefore keeping their physiological roles.

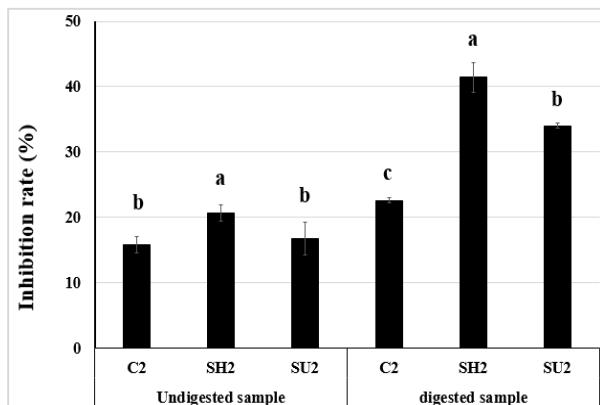


Fig. 4. Inhibition rate (%) of *L. acidophilus* in white soft cheese and white soft cheese-like products prepared using milk fat, shortening oil, and cocoa butter substitute oil.

Values are means \pm SD of three independent replicates. Column charts with different superscripts are significantly different ($p < 0.05$). C2: cheese samples prepared with milk fat and containing *L. acidophilus*. SH2: cheese samples prepared with shortening oil and containing *L. acidophilus*. SU2: cheese samples prepared with cocoa butter substitute oil and containing *L. acidophilus*.

Given the above, some strategies should be considered to protect the probiotic viability in white soft cheese and white soft cheese-like products such as microencapsulation, selecting the resistant lactic acid bacteria, and using the more suitable fat type and content.

CONCLUSION

Probiotic viability has been decreased by the progress of storage time and after *in vitro* digestion. Thus, bacterial viability can be successfully maintained by following some recent techniques such as microencapsulation. Moreover, probiotic viability in cheese is strain-dependent. So, selecting suitable probiotic strains is of great importance for keeping higher viable counts and thus can demonstrate their physiological functions.

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استخدام طريقة الهضم المعملي في تقييم حيوية *Lactobacillus spp.* في المنتجات الشبيهة بالجبن الإيبيض الطري

علياء على درويش*

قسم بحوث الالبان، معهد بحوث تكنولوجيا الأغذية، مركز البحوث الزراعية، جيزه، 12613 ، مصر

في هذه الدراسة تم انتاج ثلاثة عينات جبن محتوية على بكتيريا *L. casei* (C1)، زيت الشورتنينج (SH1)، و زيت بديل زبدة الكاكاو (SU1) وأيضاً تم انتاج ثالث عينات جبن اخرى محتوية على بكتيريا *L. acidophilus* (C2)، زيت الشورتنينج (SH2)، و زيت بديل زبدة الكاكاو (SU2). تم تقييم الجبن من حيث التركيب الكيميائي، قيم pH و حيوية بكتيريا *L. casei* and *L. acidophilus* (C1, C2, SH1, SH2) قبل الهضم المعملي وبعد الهضم (30 يوماً). اثبتت النتائج ان حيوية *Lactobacillus spp.* كانت تعتمد على السلالة حيث لوحظ ان قيم pH انخفضت تدريجياً بمدورة الوقت (عينات الجبن المحتوية على *L. casei* له حيوية أعلى من بكتيريا *L. acidophilus*). كما وجد ان حيوية *L. casei* و *L. acidophilus* في عينات *Lactobacillus spp.* كانت تختلف على اساس اعداد *L. casei* في العينة. اظهرت النتائج ان *L. acidophilus* يخضع بشدة من حيوية بكتيريا *Lactobacillus* ووجد ان *L. acidophilus* كانت السلالة الاكثر تأثيراً على *L. casei*. اظهرت النتائج التأثير الكبير لنوع الدهن المستخدم في تصنيع الجبن على حيوية بكتيريا *Lactobacillus* حيث لوحظت اعلى اعداد بكتيرية حية في عينات الجبن المحتوية على دهن اللين (C1, and C2). ايضاً، لوحظ ان معدل التثبيط (%) تأثر بشدة بعملية الهضم المعملي، نوع الدهن، وسلالة *Lactobacillus* المستخدمة. طبقاً لذلك، تم تسجيل اعلى معدل تثبيط لـ *L. acidophilus* في الجبن المهجوم المحترى على الشورتنينج وزيت بديل زبدة الكاكاو. وبالتالي، أشارت نتائج الدراسة الحالية إلى أن دهن اللين هو أنساب أنواع الدهون في الحفاظ على حيوية بكتيريا البروبيوتيك في الجبن.